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Identification and physiological significance of mycorrhizal associations of apple (Malus domestica Borkh.)

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IDENTIFICATION AND PHYSIOLOGICAL SIGNIFICANCE OF MYCORRHIZAL ASSOCIATIONS OF APPLE (MALUS DOMESTICA BORKH.)

Iowa State University PH.D. 1983

Identification and physiological significance of mycorrhizal associations of apple (*Malus domestica* Borkh.)

by

Diane Doud Miller

A Dissertation Submitted to the
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GENERAL INTRODUCTION

Explanation of Dissertation Format

The 1981 revision of the Iowa State University Graduate College Thesis Manual describes an alternate dissertation format whereby manuscripts for submission to scholarly journals may be placed between a general introduction and an overall summary and discussion. This dissertation is written following those guidelines.

Description of North Central Region Cooperative Project 140

This research on apple tree mycorrhizae was undertaken as a portion of the Iowa State University contribution to the North Central Region Cooperative Project 140 (NC-140) "Scion/Rootstock and Interstem Effects on Apple Tree Growth and Fruiting". The NC-140 project is oriented toward increasing fruit production efficiency by analyzing selected aspects of fruit tree genetics and physiology. Factors which influence utilization of soil nutrient resources bear investigation. These include biological associations such as the root: fungus symbiosis termed mycorrhiza. My research was to examine mycorrhizal associations of apple, to identify the fungal symbionts, and to evaluate the role of the symbiosis in plant mineral nutrition and stress adaptation.

The rootstock planting sampled in the survey portion of this

study was established by the NC-140 group in spring 1980 at sites in 18 states. The purpose of the planting was to enable comparative evaluation of selected rootstocks in the various environmental regimes. The planting provided an opportunity to obtain soil samples documented with site history including soil type, soil fertility, and past vegetation, and root samples documented with uniform nursery propagation. The samples are integrally involved in all sections of this dissertation. Analyses of the samples are presented in Section I of this dissertation. Section II reports differential colonization and efficacy of some survey collected fungal species in association with apple seedlings.

This study is indebted to the members of the NC-140 committee for their cooperation, both in allowing their sites to be sampled, and in providing background information about each site. During the course of this study, the author was supported by a graduate research assistantship provided through the Department of Horticulture. Research support was provided through Hatch Act funds and through a $1500 grant over two years from the Rootstock Research Foundation of the International Dwarf Fruit Tree Association.

Introduction to Mycorrhizae

A mycorrhiza (plural= mycorrhizae) consists of three components: the root cells of the host plant, the fungal hyphae within or among these root cells, and the associated fungal hyphae extending outside

of the root into the soil (Smith, 1974). There are at least five distinct types of mycorrhizal associations. These had been classified as ectomycorrhizae, vesicular-arbuscular mycorrhizae, ericoid mycorrhizae, arbutoid mycorrhizae, and orchidaceous mycorrhizae. The common feature among types of mycorrhizae is the non-pathogenicity of soil fungi in symbiosis with plant roots or rhizoids. The types differ in species of plants and fungi involved, and types of fungal structures formed in the association (Smith, 1980). There are many reviews of these types of mycorrhizae (Nicolson, 1967; Gerdemann, 1968; Went and Stark, 1968; Mosse, 1973b; Meyer, 1974; Gerdemann, 1975; Trappe and Fogel, 1977; Hayman, 1978; Pritchett, 1979; Smith, 1980).

Vesicular-arbuscular mycorrhizae (VAM) are the most widespread type of mycorrhizae both in number of plant species involved and in geographic distribution (Gerdemann, 1975). VAM are ancient and probably facilitated the evoluation of vascular plants (Baylis, 1974; Malloch, et al., 1980; Pirozynski, 1981). Most cultivated plants of the temperate zone, annuals and perennials (including Malus) form VAM (Gerdemann, 1975) and it is this type of mycorrhizae which will be discussed here.

There are three main factors which influence mycorrhiza formation: the host plant, the endophyte, and the environment (especially the soil). The presence of fungal colonization, the significance of colonization to plant growth, and the efficiency of the symbiosis in nutrient uptake can vary widely with these factors

and their interactions.

The influence of host, endophyte, environment, and their interactions will be discussed, with emphasis on pertinence to apple tree growth.

The Plant Host

Research on VAM and its significance to plant growth has focused on annual plants, not woody perennials such as fruit trees. Woody perennials which form ectomycorrhizal associations (e.g., Pinus, Quercus) also have been studied widely. Consequently, little is known regarding the influence of VAM on the performance of deciduous fruit trees.

One might develop a hypothesis regarding the role of VAM with apple trees by examining the habitat, ecological niche, and companion species of uncultivated Malus. The genus Malus is composed of approximately 15 species, four of which are native to North America (M. fusca, M. ioensis, M. angustifolia, and M. coronaria) (Westwood, 1978). Two species are indigenous to Europe (including M. pumila, which undoubtedly is the parent of most of our domestic cultivars) and the others are from Asia (Bailey, 1911; Westwood, 1978). Regardless of origin, all Malus species are regarded as intermediate pioneering species (Preston, 1976). The species generally grow best on upland sites, will not tolerate wet sites, are not shade tolerant, and are not large, long-lived trees (Preston, 1976). Examples of companion

species in natural habitats in the Midwestern United States are Prunus serotina (black cherry), Sassafras albidum (sassafras), Acer rubrum (red maple), Morus alba (mulberry), and Populus sp. (poplars).

Woody species generally are considered to be more mycorrhizae-dependent than herbaceous species (Trappe and Fogel, 1977). Woody species which compose climax forests in the temperate zone are usually ectomycorrhizal and the association is considered essential to facilitate the intense nutrient cycling of the ecosystem (Malloch, et al., 1980). Woody pioneering species are commonly endomycorrhizal and reports on the mycorrhizae-dependence of this ecosystem are scarce (St. John and Coleman, 1983).

Mycorrhizal colonization is very widespread in natural ecosystems. Ancestrial "native" plants have been reported to be more mycorrhizae-dependent than progenic "crop" plants which seldom exhibit absolute mycorrhizae-dependence (St. John and Coleman, 1983).

The Malus species involved in horticultural fruit production is commonly designated Malus domestica Borkh. (Tukey, 1964). It was thus designated to emphasize that selection and domestication over hundreds of years have obscured its exact origin (Westwood, 1978). A great deal of genetic diversity exists in cultivated Malus, and its ability to adapt to diverse climates makes it the most widely planted fruit tree of the temperate zone (Westwood, 1978). This genetic potential is under-utilized as the majority of commercial production is accounted for by only five cultivars grafted onto an equally limited number of rootstock clones (Westwood, 1978). It is the rootstocks

and, in particular, the root systems which are of special interest here.

Root systems are difficult to study in situ and for this reason much less is known about them than shoot systems. However, apple roots have been studied more than roots of any other woody perennial of the temperate zone. Apple root research was stimulated by the work of Hatton at East Malling Research Station in England in the 1910's who sorted out a series of size controlling rootstocks. He demonstrated that vigor, productivity, fruit quality, and other characteristics could be altered and controlled by these rootstocks (Hatton, 1916; Hatton, 1919; Hatton, 1927; Hatton, 1930; Hatton,1939). Root observation chambers were consequently established at East Malling and a series of root studies initiated by Rogers (1939), Coker (1958), and Head (1966, 1967, 1968). The question "why are dwarf rootstocks dwarfing?" has never been answered but apple root growth has been well characterized.

Apple root systems are composed of roots of a variety of ages, diameters, and distributions (Rogers, 1939). All young apple roots are white and succulent with very short (0.025 to 0.075 mm) root hairs (Rogers, 1939). After 1 to 4 weeks the root cortex begins to turn brown and root hairs shrivel (Rogers, 1939). The cortex is sloughed and either the root dies and disintegrates or suberization and secondary thickening occur and the root becomes part of the perennial root system bearing new roots as laterals (Rogers, 1939). An estimated one-half of the dry matter produced by extension roots is

shed soon after it is produced (Rogers, 1968).

Apple root distribution is dependent upon planting density (Atkinson, 1980). Atkinson (1980) reported that at low planting densities the root system was composed of mostly horizontal roots with few vertical roots. As planting density was increased, the majority of roots were vertical and a higher proportion of roots were present deeper in the profile (Atkinson, et al., 1976). Cover crops such as grass also can affect root distribution, usually resulting in an increased percentage at lower depths (Atkinson, 1980). When trees were grown in an herbicide strip, the majority of root growth took place within the strip and few roots penetrated the grassed alleyways (Atkinson, 1977).

Peak periods of apple root growth were found to occur in spring-early summer and late summer-autumn (Head, 1967). In mid-summer, when the majority of shoot growth occurred, few new roots were produced. Heavy cropping and pruning of trees created strong carbon sinks in the shoot system and reduced root growth (Atkinson, 1980).

The relationship between root growth and root activity in nutrient uptake has been studied little in fruit trees. Atkinson (1974), working with 2-year-old trees, assayed root activity via uptake of ^{32}P tracer. He concluded that root activity in phosphorus uptake was greatest at the 0 to 30 cm depth during the seasonal peak periods of white root production. This corresponded closely with the seasonal fluxes in phosphorus concentrations of apple tissues reported by Mason and Whitfield (1960).

Phosphorus is the element most limiting to plant growth in natural ecosystems due to its low availability (Ozanne, 1980). It readily complexes with iron and aluminum at low pH and with calcium at high pH (Ozanne, 1980). It is also the element whose uptake is most typically increased by mycorrhizae (Tinker, 1980).

There is considerable circumstantial evidence suggesting a significant role of mycorrhizae in phosphorus uptake of field-grown apple trees. Apple trees have low root densities and small total root length compared with other crop plants (Atkinson and Wilson, 1980), and short root hairs (Rogers, 1939), yet phosphorus deficiency in the field is rare (Greenham, 1976). Fruit trees are efficient at phosphorus uptake, often growing well in low phosphorus soils where other crop plants show deficiencies (Lilleland, 1932), and rarely responding to phosphorus fertilization (Greenham, 1976).

There is speculation that mycorrhizae-dependency of perennials varies with tree age (St. John and Coleman, 1983). Dependence on mycorrhizae for phosphorus uptake is probably greatest in seedlings as there is less storage tissue and less extensive root to living biomass ratios (St. John and Coleman, 1983).

Phosphorus is the major nutrient whose uptake is increased by mycorrhizae but the uptake of other rather immobile elements such as sulfur (Gray and Gerdemann, 1973; Rhodes and Gerdemann, 1978a; Rhodes and Gerdemann, 1978b), zinc (Benson and Covey, 1976; Jensen, 1982), and copper (Jensen, 1982) has been increased.

It is unfortunate that, until recently, fruit tree nutrition

research and mycorrhizae research have not been conducted jointly. The fact that fruit tree roots form vesicular-arbuscular mycorrhizae is not a recent discovery. Boulet (1910) first histologically characterized the mycorrhizae of apple, pear, plum, peach, cherry and quince rootlets. Smith (1930) surveyed roots of apple, pear, plum, peach, and cherry in the Ann Arbor, Michigan, area in 1928 and reported all species examined were endomycorrhizal. He found no ectomycorrhizal associations with these species. Bouwens (1937) found VAM in apple, pear, plum, peach, cherry, quince, and mulberry and tried unsuccessfully to isolate in pure culture the fungal endophyte. Rogers (1939), at the East Malling root chambers, described and photographed soil fungi associated with young apple roots but was unsure of the relationship. Mosse (1956) reported the establishment of VAM in aseptically grown apple seedlings following inoculation with spores and sporocarps of an Endogone species (now known to be Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe). Otto (1962a, 1962b, 1963) characterized the mycorrhizae of apple seedlings grown in various habitats by categories of frequency, intensity, and persistence. He could not attribute any damaging or stimulating effect of VAM to apple tree growth. Meyer (1973) reviewed ectomycorrhizae and listed Malus as a genus which may be ectomycorrhizal in the wild but is endomycorrhizal in cultivation.

The first evidence of the potential benefit of mycorrhizae to apple tree growth was reported by Mosse (1957). She found increased growth and potassium, iron, and copper concentrations in mycorrhizal

as compared to non-mycorrhizal apple seedlings. Recently, several greenhouse studies have reported growth stimulation, with or without associated increases in plant nutrient concentrations, of mycorrhizal apple seedlings grown under low phosphorus regimes (Covey, et al., 1981; Plenchette, et al., 1982; Plenchette, et al., 1983a; Plenchette, et al., 1983b; Hoepfner, et al., 1983). A VAM-induced growth response in low zinc soil has been noted with apple seedlings (Benson and Covey, 1976) and with peach seedlings (Gilmore, 1971; LaRue, et al., 1975; Lambert, et al., 1979b). Greene, et al. (1982) reported a growth stimulation but no ectomycorrhizal association or increased nutrient uptake from inoculating apple rootstocks with the ectomycorrhizal fungus, Pisolithus tinctorius, at field planting time. Whether field inoculation with endomycorrhizal fungi can stimulate a growth response in apple trees remains unclear and will be dealt with further in the section on the endophyte.

The role of VAM in plant disease, especially specific replant disease of apple, is being researched but is not yet understood and will not be reviewed here.

The Endophyte

The fungi that associate with plant roots to form VAM in the temperate zone are species of four genera in the Endogonaceae- Glomus, Sclerocystis, Gigaspora, and Acaulospora (order Endogonales, class Zygomycotina) (Gerdemann and Trappe, 1974). Genera have been

separated on the basis of asexual spore formation and spore morphology. Sexual cycles, if such exist, remain unknown. The fungi are obligate symbionts with plant hosts and have not, as yet, been grown in pure culture. Consequently, although these fungi are common and widely distributed soil fungi, they have not been recognized as such until recently.

Glomus spores are termed chlamydospores, are borne in or not in sporocarps, and are formed terminally on an undifferentiated hypha (Gerdemann and Trappe, 1974). Sclerocystis spores also are considered to be chlamydospores and are related closely to Glomus, differing from Glomus in the orderly arrangement of spores around a central plexus of sterile hyphae in a sporocarp (Gerdemann and Trappe, 1974). Gigaspora spores are termed azygospores, are nonsporocarpic, and are borne on a hyphal suspensor (Gerdemann and Trappe, 1974). Acaulospora spores also are termed azygospores, are non-sporocarpic, and are borne on a hypha which terminates in a thin-walled mother vesicle (Gerdemann and Trappe, 1974).

Within genera, species have been delineated by such characteristics as number and thickness of spore walls, wall ornamentation, spore dimensions, color, contents, reaction to Meltzer's reagent, and hyphal mantles (Gerdemann and Trappe, 1974). Germination characteristics and characteristics of mycorrhizae formed also have been suggested as diagnostic tools (Trappe and Schenck, 1982).

Many taxonomic keys of these genera and their species have been

published (Nicolson and Gerdemann, 1968; Gerdemann and Trappe, 1974; Tandy, 1975; Hall, 1977; Nicolson and Schenck, 1979). Recently, a dichotomous key (Hall and Fish, 1979) and synoptic keys (Schenck and Smith, 1982; Trappe, 1982) have been developed.

It remains difficult, but essential, to correctly identify the species with which one is working. There are complications such as intergrading taxa and little information is available on within species (isolate) variation.

As interest in these fungi has increased due to their involvement in mycorrhizal assocations, more species have been described. When the family Endogonaceae was monographed in 1974 (Gerdemann and Trappe), 19 species of Glomus, 4 species of Sclerocystis, 5 species of Gigaspora, and 2 species of Acaulospora were described. Presently, 47 species of Glomus, 8 species of Sclerocystis, 19 species of Gigaspora, and 9 species of Acaulospora have been described (Tables 1-4). Trappe (1982) has predicted that the total number of described species will approach 200 by 1990 as mycorrhizae in other parts of the world are investigated.

There are three other genera in the Endogonaceae- Endogone, Entrophosphora, and Glaziella (Gerdemann and Trappe, 1974). Endogone, the type genus, is not know to form VAM (Gerdemann and Trappe, 1974). Entrophosphora may form VAM but this has not yet been verified (Ames and Schneider, 1979). There is only one species of Glaziella (Gerdemann and Trappe, 1974). It is from the tropics and has been little studied. Two genera formerly placed in this family, Modicella

Table 1. Described species of Glomus and (reference)

G. aggregatum Schenck & Smith (Schenck and Smith, 1982)

G. albidum Walker & Rhodes (Walker and Rhodes, 1981)

G. boreale (Thaxter) Trappe & Gerd. (Thaxter, 1922; Gerdemann and Trappe, 1974)

G. caledonium (Nicol. & Gerd.) Gerd. & Trappe (Nicolson and Gerdemann, 1968; Gerdemann and Trappe, 1974)

G. canadense (Thaxter) Trappe & Gerd. (Thaxter, 1922; Gerdemann and Trappe, 1974)

G. claroideum Schenck & Smith (Schenck and Smith, 1982)

G. clarum Nicol. & Schenck (Nicolson and Schenck, 1979)

G. constrictum Trappe (Trappe, 1977)

G. convolutum Gerd. & Trappe (Gerdemann and Trappe, 1974)

G. epigaeum Daniels & Trappe (Daniels & Trappe, 1979)

G. etunicatum Becker & Gerd. (Becker and Gerdemann, 1976)

G. fasiculatum (Thaxter sensu Gerd.) Gerd. & Trappe (Thaxter, 1922; Gerdemann, 1965; Gerdemann and Trappe, 1974)

G. fecundisporum Schenck & Smith (Schenck and Smith, 1982)

G. flavisporum (Lange & Lund) Trappe & Gerd. (Lange and Lund, 1954; Gerdemann and Trappe, 1974)

G. fragile (Berk. & Br.) Trappe & Gerd. (Gerdemann and Trappe, 1974)

G. fuegianum (Speg.) Trappe & Gerd. (Gerdemann and Trappe, 1974)

G. fulvum (Berk. & Br.) Trappe & Gerd. (Gerdemann and Trappe, 1974)

G. geosporum (Nicol. & Gerd.) Walker (Nicolson and Gerdemann, 1968; Walker, 1982)

G. gerdemanni Rose, Daniels, & Trappe (Rose, Daniels, and Trappe, 1979)

G. halonatum Rose & Trappe (Rose and Trappe, 1980)

Table 1 continued

G. intraradices Schenck & Smith (Schenck and Smith, 1982)

G. infrequens Hall (Hall, 1977)

G. invermaium Hall (Hall, 1977)

G. lacteum Rose & Trappe (Rose and Trappe, 1980)

G. leptotichum Schenck & Smith (Schenck and Smith, 1982)

G. macrocarpum Tul. & Tul. (Tulasne and Tulasne, 1845; Gerdemann and Trappe, 1974)

G. magnicaule Hall (Hall, 1977)

G. melanosporum Gerd. & Trappe (Gerdemann and Trappe, 1974)

G. microcarpum Tul. & Tul. (Tulasne and Tulasne, 1845; Gerdemann and Trappe, 1974)

G. monosporum Gerd. & Trappe (Gerdemann and Trappe, 1974)

G. mosseae (Nicol. & Gerd.) Gerd. & Trappe (Nicolson and Gerdemann, 1968; Gerdemann and Trappe, 1974)

G. multicaule Gerd. & Bakshi (Gerdemann and Bakshi, 1976)

G. occultum Walker (Walker, 1982)

G. pallidum Hall (Hall, 1977)

G. pubescens (Sacc. & Ell.) Trappe & Gerd. (Thaxter, 1922; Gerdemann and Trappe, 1974)

G. pulvinatum (Henn.) Trappe & Gerd. (Thaxter, 1922; Gerdemann & Trappe, 1974)

G. radiatum (Thaxter) Trappe & Gerd. (Thaxter, 1922; Gerdemann and Trappe, 1974)

G. reticulatum Bhatt. & Muk. (Bhattachargee and Mukerji, 1980)

G. scintillans Rose & Trappe (Rose and Trappe, 1980)

G. segmentatum Trappe, Spooner & Ivory (Trappe, 1979)

G. tenerum Tandy (Tandy, 1975)

Table 1 continued

G. tenue (Greenall) Hall (Hall, 1977)
G. tortuosum Schenck & Smith (Schenck and Smith, 1982)
G. tubaeforme Tandy (Tandy, 1975)
G. vesiculiferum (Thaxter) Gerd. & Trappe (Thaxter, 1922; Gerdemann and Trappe, 1974)

Table 2. Described species of Sclerocystis and (reference)

S. clavispora Trappe (Trappe, 1977)
S. coccogena (Pat.) von Hohn (Thaxter, 1922)
S. coremioides Berk. & Br. (Gerdemann and Trappe, 1974)
S. dusii (Pat.) von Hohn (Thaxter, 1922; Gerdemann and Trappe, 1974)
S. microcarpus Iqbal & Bushra (Iqbal and Perveen, 1980)
S. pakistanica Iqbal & Bushra (Iqbal and Perveen, 1980)
S. rubiformis Gerd. & Trappe (Gerdemann and Trappe, 1974)
S. sinuosa Gerd. & Bakshi (Gerdemann and Bakshi, 1976)

Table 3. Described species of Gigaspora and (reference)

Gi. albida Schenck & Smith (Schenck and Smith, 1982)

Gi. alborosea Ferr. & Herr. (Ferrer and Herrera, 1981)

Gi. aurigloba Hall (Hall, 1977)

Gi. calospora (Nicol. & Gerd.) Gerd. & Trappe (Gerdemann and Trappe, 1974)

Gi. coralloida Trappe, Gerd. & Ho (Gerdemann and Trappe, 1974)

Gi. gigantea (Nicol. & Gerd.) Gerd. & Trappe (Gerdemann and Trappe, 1974)

Gi. gilmorei Trappe & Gerd. (Gerdemann and Trappe, 1974)

Gi. gregaria Nicol. & Schenck (Nicolson and Schenck, 1979)

Gi. heterogama (Nicol. & Gerd.) Gerd. & Trappe (Gerdemann and Trappe, 1974)

Gi. margarita Becker & Hall (Becker and Hall, 1976)

Gi. minuta Herr. & Ferr. (Ferrer and Herrera, 1981)

Gi. nigra Redhead (Nicolson and Schenck, 1979)

Gi. pellucida Nicol. & Schenck (Nicolson and Schenck, 1979)

Gi. reticulata Koske, Miller & Walker (Koske, Miller and Walker, 1983)

Gi. rosea Nicol. & Schenck (Nicolson and Schenck, 1979)

Gi. savannicola Herr. & Ferr. (Ferrer and Herrera, 1981)

Gi. tricalypta Herr. & Ferr. (Ferrer and Herrera, 1981)

Table 4. Described species of Acaulospora and (reference)

A. bireticulata Rothwell & Trappe (Rothwell and Trappe, 1979)
A. elegans Trappe & Gerd. (Gerdemann and Trappe, 1974)
A. foveata Trappe & Janos (Janos and Trappe, 1982)
A. gerdemannii Schenck & Nicol. (Nicolson and Schenck, 1979)
A. laevis Gerd. & Trappe (Gerdemann and Trappe, 1974)
A. scrobiculata Trappe (Trappe, 1977)
A. spinosa Walker & Trappe (Walker and Trappe, 1981)
A. trappei Ames & Lind. (Ames and Linderman, 1976)
A. tuberculata Janos & Trappe (Janos and Trappe, 1982)

and Complexipes, do not belong. Modicella forms sporangia and is saprobic (Trappe, 1982). Complexipes is a stage of a Discomycete in the family Pyronemataceae (Danielson, 1982).

A mycorrhiza is established when a fungal hypha contacts, penetrates, and colonizes a plant root (Hayman, 1983). The hypha may be a germ tube from a resting spore or it may be growing from a previously established mycorrhiza (Hayman, 1983). The hypha forms an appressorium and penetrates through or between epidermal cells (Carling and Brown, 1982). Then, colonization of the root cortex occurs without apparent damage to the integrity of the cortical cells (Carling and Brown, 1982). After initial colonization, the fungus may be present as intracellular, intercellular, or external hyphae, vesicles (terminal hyphal swellings present intra- or intercellularly), arbuscules (finely branching hyphae which form in cortical cells), and spores (quiescent structures) (Smith, 1974). Considerable detail of the ultrastructure of the association has been published (Cox and Sanders, 1974; Kinden and Brown, 1975a; Kinden and Brown, 1975b; Kinden and Brown, 1976; Scannerini and Bonfante-Fasolo, 1979). Buwalda, et al (1982) suggested that after initial colonization, infections spread primarily along roots instead of randomly via contact among radiating hyphae and roots. A mathematical model of colonization in clover (Trifolium) has been developed by Smith and Walker (1981).

VAM fungi exhibit very limited independent growth (Warner and Mosse, 1980) so studies of these fungi must be conducted in

conjunction with a host plant. For agricultural researchers, the response of the host plant to colonization has been easier to quantitate and of more economic interest than the activity of the fungi. Many perplexing questions have arisen from these studies and few generalities have resulted. There is little apparent host specificity with these fungi and most species can colonize a broad range of host plants (Carling and Brown, 1982). Any one fungal species has not colonized similarly, and stimulated a uniform growth response, in all host species tested (Gaunt, 1978; Hall, 1978; Azcon and Ocampo, 1981; Pope, et al., 1983). Conversely, with any one particular host species or cultivar within species, not all fungal species have performed similarly (Jensen, 1982; Daft and Hogarth, 1983; Pope, et al., 1983).

Mycorrhizal fungi are indigenous to agricultural fields and usually several different fungal species have been reported to cohabitate simultaneously (Mosse and Bowen, 1968; Schenck and Hinson, 1971; Sutton and Barron, 1972; Abbott and Robson, 1977; Ames and Linderman, 1977; Herskowitz and Estey, 1978; Molina, et al., 1978; Hayman and Stovold, 1979; Schenck and Kinloch, 1980). It may be that, in field situations, the maximum benefit from the association is inherent and research will just facilitate a better understanding of the value of the symbiosis. There are instances in fruit production (and other crop production) when inoculation with mycorrhizal fungi would be beneficial to plant establishment and growth, especially if particularly effective species were known. The instances include: in

fumigated soils, in acclimating tissue-cultured plants to soil environments, in nursery propagation of rootstocks, and in soils which (due to agricultural chemicals or other factors) do not contain endophytes effective with apple. In a field study, Plenchette, et al. (1981) compared preinoculated plants with controls that became mycorrhizal naturally after outplanting, and attributed growth differences to the species of fungus. However, the study was not properly replicated and compared trees in different physiological stages.

The simplest explanation of why fungi and host plants differ in response to the mycorrhizal association is related to root density. The coarser the root system, and the fewer the root hairs, the greater the dependence upon mycorrhizae (e.g. citrus trees are known to be very mycorrhizae dependent while grasses are much less dependent) (Baylis, 1970; Baylis, 1972). The various fungi likely extend the "mycorrhizosphere" to different lengths via external hyphae (Menge, 1983). The interaction of the density of the root system and the amount of external hyphae theoretically should explain the growth response pattern of the host (Hayman, 1982). This is complicated by genetic differences in phosphorus requirements among host plant species and even among cultivars within species (Shear and Faust, 1980). Above the phosphorus threshold level (i.e. concentration where phosphorus is not growth limiting), no growth response due to additional phosphorus can be observed. Unfortunately, quantifying root geometry, roots, root hairs, and external hyphae at a given time

or during a period of time is very difficult.

When phophorus (and other immobile elements) are removed from the soil solution, due to their slow diffusion coefficients, a zone of depletion occurs around the root (Ozanne, 1980). Consequently, the process of phosphorus extraction relies heavily on exploration of the soil by plant roots, root hairs, and hyphae of mycorrhizal fungi to increase the physical volume of soil explored rather than on diffusion of phosphorus in soil solution to the plant root (Tinker, 1980). There is no strong evidence that mycorrhizal fungi have access to phosphorus in any form not accessible to the root (Hayman and Mosse, 1972).

The intensity of fungal colonization within the root may or may not be correlated with a host plant growth response (Carling and Brown, 1982; Graham, et al., 1982b). Bethlenfalvay, et al. (1982) suggested that the amount of external hyphae which a fungus produced was responsible for differences in growth stimulation of host plants. As it is the external hyphae which scavenge soil phosphorus and other nutrients, it seems plausible that fungi which produce large amounts of external hyphae would be superior symbionts (Graham, et al., 1982b). The ratio between external and internal hyphae production with any fungal species is unknown. Fungi which produce little external hyphae but extensive internal hyphae or vesicles may be parasitic on the host plant (Bethlenfalvay, et al., 1982). Hayman (1983) has suggested that it is differences in arbuscule formation and breakdown that separate superior and inferior symbionts. In field

situations, plants are frequently colonized by several endophytes (Hayman, 1982; Daft and Hogarth, 1983) and the competitive interactions that occur in these situations are not understood. Hayman (1982) contended that apparent ineffectiveness of some endophytes may relate to their slowness to colonize.

Differences in efficacy of endophytes has been related to inoculum density (Daniels, et al., 1981). Inoculum potential may be influenced not only by spore number but by spore germination, maturity, dormancy, type, rate of growth to root, colonizaton ability, and interactions with other microbes (Menge, 1983). Efficacy of fungi also may be influenced by soil phosphorus level (Hayman, 1982) and phosphorus fertilizer applications (Lambert, et al., 1979a), applications of agricultural chemicals (Smith, 1978; Pope and Holt, 1981; Tommerup and Briggs, 1981) and hyperparasites (Ross and Ruttencutter, 1977; Schenck and Nicolson, 1977; Daniels and Menge, 1980; MacDonald and Chandler, 1981).

In the mycorrhizal symbiosis, the fungi are provided by the plant both with a growing space and with a carbohydrate source. It has been suggested that fungal symbionts vary as sinks for photosynthate and this also may be a factor in apparent efficacy (Coleman, 1976). With apple, most root growth occurs (Head, 1967) and most phosphorus is taken up (Atkinson, 1974), when the most carbohydrates are transported to the roots (in late summer and fall) (Kandiah, 1979a; Kandiah, 1979b). At any time, the biomass of the roots may be equal to or smaller than the biomass of the shoots, but total input of

carbohydrates to the roots is larger than that to the shoots (Coleman, 1976). Mycorrhizae represent a large investment in carbohydrates and have rapid turnover (Hayman, 1983). Fungal colonization at any given time underestimates the total fungal biomass which has been supported over a growing season (Coleman, 1976). Mycorrhizal plants often contain higher shoot phosphorus concentrations than non-mycorrhizal plants of equal size and this has been attributed to the carbon drain via colonized roots (Stribley, et al., 1980).

The Environment

The third variable influencing mycorrhizae formation and function is the environment, especially the soil. Components of the environment include characteristics of the soil- fertility (in particular soluble phosphorus levels), pH, moisture, organic matter, and microfauna- as well as the above ground components such as irradiance levels and temperature (Hayman, 1982). The physical characteristics of the soil also influence plant root configuration and geometry and may alter mycorrhizae-dependence (Menge, 1983).

Characteristically, mycorrhizae are prevalent and significant to plant growth in soils which have low levels of soluble phosphorus (Tinker, 1978). Often little root colonization is present at high phosphorus fertility, especially in experiments with potted plants (Hayman, 1982). Field fertilization with phosphorus is inefficient, as Mosse (1973b) estimated 75% of that applied reverts to plant

unavailable forms during the year of application. Menge (1983) considered mycorrhizae to be "biotic fertilizers" because they increase the efficiency of phosphorus fertilization by extending the absorption zones. Barea, et al. (1980) reported effective endophytes stimulated Medicago sativa growth in a similar manner to, but more economically than, the addition of soluble phosphorus fertilizer. Mycorrhizal and non-mycorrhizal roots have access to the same forms of phosphorus and increased uptake of phosphorus by VAM is due to the physical characteristic of additional absorption surface (Hayman and Mosse, 1972; Ross and Gilliam, 1973).

The mechanisms of inhibition of mycorrhizal colonization at high phosphorus levels are not understood. It has been suggested that plant phosphorus levels, not soil phosphorus levels, control colonization (Menge, et al., 1978c; Jasper, et al., 1979) possibly via membrane-mediated root exudation (Ratnayake, et al., 1978; Graham, et al., 1982a). Exudation from root hairs of apple roots has been described in detail but the function of the phenomenon is not understood (Head, 1964).

Mosse (1973b) contended there was more specificity between soil and fungal species than between fungal species and plant host. Hayman (1982) noted that in agricultural situations the selective pressure on VAM fungi populations may be toward infectivity rather than effectivity as plants are rarely obligately dependent on the symbiosis for nutrient uptake.

Moderate phosphorus fertilization of a pasture soil in Australia

over several years increased the number of VAM fungal spores compared to nonfertilized or heavily fertilized soil but did not affect the infectivity or effectivity of the indigenous endophytes (Porter, et al., 1978). Hayman (1970) reported field spore numbers were steady from December to June, peaked in July, and began decreasing in September.

Soil pH is believed to affect the symbiosis. Laboratory studies have shown the germination of Glomus mosseae spores was favored by neutral to alkaline conditions (Green, et al., 1976). Glomus mosseae was reported to be a superior symbiont with soybean at pH 6.2 while Gigaspora gigantea was superior at pH 5.1 (Skipper and Smith, 1979). Glomus mosseae has been reported to colonize hosts effectively only in soils with pH greater than 5.5 (Mosse, 1972).

There has been limited evidence that mycorrhizae assist with plant water uptake (Safir, et al., 1971; Safir, et al., 1972). The moisture content of the soil may influence survival of the fungi and their ability to colonize a host (Slankis, 1974). In waterlogged soils, oxygen deficiency limits both root growth and VAM fungi growth (Slankis, 1974).

Organic matter addition to soil is purported to stimulate VAM and Hayman (1982) hypothesized that organic matter may be a maintenance substrate for the fungi. Saprophytic survival of these fungi has not been shown unequivocally.

The competitive interactions that occur within and among species of mycorrhizal fungi and between VAM fungi and other soil organisms is

little understood. How much predation of mycorrhizal fungi occurs by hyperparasites, nematodes, or soil insects and the ecological consequences are unknown. Present knowledge of the interactions of VAM fungi with plant pathogens has been reviewed by Dehne (1982).

Air temperature and light effects on spore germination and root colonization have been inconsistent among studies and generalizations are limited. Schenck and Schroder (1974) reported Gigaspora gigantea formed maximum arbuscules within soybean roots at 30 C. External hyphae formation was greatest at 28 to 34 C and most spores were formed at 35 C. Furlan and Fortin (1973) reported that root colonization followed a characteristic sigmoid curve with each phase- lag, exponential, and plateau- occurring quicker at high temperatures (21/26 C, night/day). At low temperature (11/16 C, night/day) a parasitic decrease in onion plant growth occurred. Schenck, et al. (1975) reported maximum spore germination of two Gigaspora species (Florida isolates) at 34 C while a Washington isolate of Glomus mosseae exhibited maximum germination at 20 C. Spore germination, in all cases, was increased in the absence of light.

Daniels and Trappe (1980) reported soil temperature, moisture and pH influenced germination of Glomus epigaeus spores. Maximum germination occurred at field capacity or above moisture levels, 18 to 25 C, and pH 6 to 8. They believed conditions for optimum spore germination paralleled conditons for optimum host plant growth.

Light and temperature of both air and soil appear to interact with soil phosphorus level to influence mycorrhizae formation.

Graham, et al., (1982a) reported that at high phosphorus levels, decreased light decreased VAM formation but at low phosphorus levels, decreased light did not decrease VAM formation. Increasing soil temperature, regardless of soil phosphorus level, increased VAM formation. Furlan and Fortin (1977) found spore production increased with light level but colonization was more rapid and percentage colonization greater at lower light levels. Johnson (1976) reported shade-tolerant forest species had less fungal colonization when grown in heavy shade as compared to full sun over a range of soil phosphorus levels.

From an agricultural input standpoint, the significance of mycorrhizae to crop production remains unquantitated (Rhodes, 1980). It is known that, with citrus, mycorrhizae are required for optimum plant performance if there is less than 34 ug/g phosphorus in the soil or if there is less than 12 ug/g zinc in the soil or if the organic matter content is less than 3% (Menge, 1983). The situation with other crops, including apple, is unknown.

SECTION 1.

GEOGRAPHIC VARIATION IN MYCORRHIZAL ASSOCIATES OF APPLE (MALUS DOMESTICA BORKH.) IN THE UNITED STATES

Geographic Variation in Mycorrhizal Associates of Apple (*Malus domestica* Borkh.) in the United States

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ABSTRACT

Vesicular-arbuscular mycorrhizae of apple (Malus domestica Borkh.) were surveyed. Soil and root samples were collected in August 1980 from 18 planting sites of the U.S.D.A. North Central Region Cooperative Project 140 apple rootstock trial. Sites varied widely in fertility. Fungal spores extracted from soil samples were used to identify species of Endogonaceae and to determine their relative abundance per site. There were no apparent patterns in geographic distribution of fungal species. A range of 3 to 8 species was found among sites. Glomus was more common in number of species and number of spores than Gigaspora, Acaulospora, or Sclerocystis. Composite pot cultures of soil from within sites yielded some species not recognized from soil sievings. Infectivity of all species isolates was tested with apple and with sorghum. A range of infective potential among soils was found via a dilution study using apple as the host plant. Survey collected roots were endomycorrhizal at all sites. There was no evidence of any ectomycorrhizal association. Percent root length colonized and intensity of root colonization per site was negatively correlated with soil zinc and (at most sites) phosphorus. There were no apparent differences in field colonization of any rootstock clone within sites. Soil infective potential estimates were well-correlated with colonization of survey collected roots. There was no evidence that any site was lacking in inoculum potential for apple.

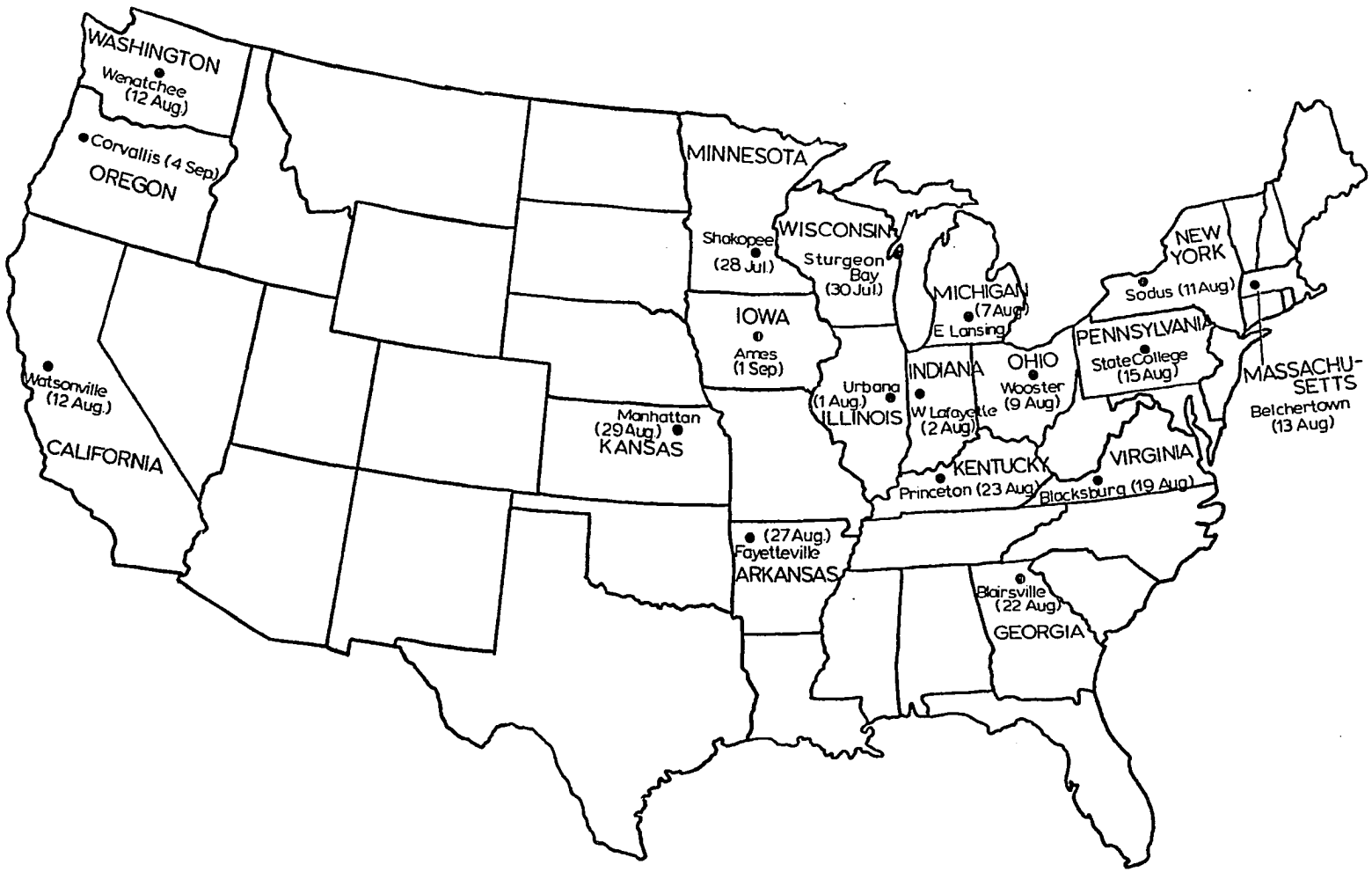
INTRODUCTION

Apple trees (Malus domestica Borkh.) are cultivated over a wider geographic range than any other temperate zone fruit tree (Westwood, 1978). Apple cultivars are propagated as scions grafted to clonal rootstocks which have been produced by stooling or mound layering (Westwood, 1978). These rootstocks have been selected for their effects on tree form and vigor. Clones that induce dwarfing are becoming increasingly important, since the trees thus formed facilitate more economic production methods.

Although much is known about the effects of rootstock genotype on fruit production and tree form (Tukey, 1964; Carlson, et al., 1970; Westwood, 1978), few studies exist in which their mycorrhizal relationships have been examined. Indeed, since early examinations showed the species to be endomycorrhizal (Boulet, 1910; Smith, 1930; Bouwens, 1937) there have been few studies (Benson and Covey, 1976; Trappe, et al., 1973) until recently, when some examinations of the interaction of mycorrhizae and nutrient regimes have been made (Covey, et al., 1981; Plenchette, et al., 1982; Plenchette, et al., 1983a; Plenchette, et al., 1983b; Hoepfner, et al., 1983).

In this study, apple mycorrhizae and their associated fungal spores were sampled from a range of locations in the United States (Figure 1). The open pot culture method of Gilmore (1968) was used to increase mycorrhizal spore populations and pure cultures were then created by inoculating Malus or Sorghum roots with spores thus obtained.

Figure 1.1 Locations of NC-140 regional apple rootstock planting sites and dates sampled, 1980


WASHINGTON
Wenatchee (12 Aug)
Corvallis (4 Sep)
OREGON
Watsonville (12 Aug.)
CALIFORNIA
MINNESOTA
Shakopee (28 Jul)
WISCONSIN
Sturgeon Bay (30 Jul)
IOWA
Ames (1 Sep)
Manhattan (29 Aug)
KANSAS
Urbana (1 Aug.)
ILLINOIS
MICHIGAN
(7 Aug)
E Lansing
INDIANA
W Lafayette (2 Aug)
OHIO
Wooster (9 Aug)
NEW YORK
Sodus (11 Aug)
PENNSYLVANIA
State College (15 Aug)
MASSACHU-SETTS
Belchertown (13 Aug)
KENTUCKY
Princeton (23 Aug)
VIRGINIA
Blacksburg (19 Aug)
(27 Aug.)
Fayetteville
ARKANSAS
Blairsville (22 Aug)
GEORGIA

MATERIALS AND METHODS

The rootstock planting sampled was established by the U.S.D.A. North Central Region Cooperative Project 140 (NC-140) in spring 1980 at sites associated with horticulture research stations in 18 states. The purpose of the planting is to enable comparative evaluation of selected rootstock performance in the various environmental regimes. Nine apple rootstock clones (Malling 9, East Malling/ Long Ashton (EMLA) 27, EMLA 9, EMLA 26, EMLA 7, Michigan Apple Clone (MAC) 9- now called MARK, MAC-24, Oregon Apple Rootstock (OAR) 1, Ottawa 3) (Carlson, 1978; Cutting and Montgomery,1973), each grafted with 'Starkspur Supreme Delicious', were present in 5 replications at each site. All trees were propagated by Oregon Rootstock, Inc., Woodburn, Oregon, selected for uniformity, and randomly designated to sites. Before those for the Iowa site were planted, samples of feeder roots of each tree were collected and preserved in formaldehyde/ acetic acid/ alcohol (FAA) in 30 ml vials for assessment of mycorrhizal status.

A record was made of the weed species present at the sampling time (late summer, 1980) and the cropping history of each site (Table 1). Four replications of the rootstock planting were sampled at each site except California and Washington where 2 replications were sampled. Three soil cores (45 cm deep) were randomly collected with a 2.5 cm diameter soil tube from within a 60 cm radius of the tree trunk and combined to give approximately 300 g of moist soil per sample

Table 1.1 Vegetation at each NC-140 regional apple rootstock planting site sampled, August 1980

SITE	CROP IN FIELD 2 YEARS BEFORE PLANTING	CROP IN FIELD 1 YEAR BEFORE PLANTING	OTHER PLANTS IN ROOT ZONE WHEN SAMPLED
Arkansas	natural vegetation	natural vegetation	*Cynodon dactylon*
California	*Lactuca sativa*	*Lactuca sativa*	*Matricaria matricarioides* *Solanum dulcamara* *Cyperus esculentus* *Amaranthus* sp.
Georgia	*Prunus persica* *Malus domestica*	*Festuca arundinacea* *Trifolium repens*	*Cynodon dactylon* *Digitaria sanguinalis* *Ambrosia* sp. *Oxalis* sp.
Illinois	*Malus domestica* *Poa pratensis*	*Poa pratensis* *Trifolium repens*	*Poa pratensis*
Indiana	*Pyrus communis*	*Sorghum sudanese* *Triticum aestivum*	*Abutilon theophrasti*
Iowa	*Citrullus lanatus* *Cucumis melo*	*Sorghum sudanese*	*Hordeum jubatum* *Poa pratensis* *Portulaca oleracea*
Kansas	*Malus domestica*	*Poa pratensis* *Digitaria sanguinalis* *Taraxacum officinale*	none

Kentucky	Malus domestica Festuca arundinacea	Festuca arundinacea	Festuca arundinacea
Massachusetts	Malus domestica Prunus persica	Agropyron repens Dactylis glomerata	Dactylis glomerata
Michigan	Festuca rubra	Festuca rubra	Festuca rubra Taraxacum officinale Trifolium repens
Minnesota	Malus domestica	Secale cereale	Secale cereale Cirsium arvense
New York	Trifolium pratense	Trifolium pratense	Trifolium pratense
Ohio	Zea mays	Poa pratensis Festuca arundinacea	none
Oregon	natural vegetation	natural vegetation	Cirsium pratense Erodium sp. Lactuca scariola
Pennsylvania	Trifolium repens Bromus inermis	Zea mays	Convolvulus arvensis Ambrosia artemisiifolia
Virginia	Trifolium pratense	Festuca arundinacea	Setaria faberii Bromus inermis
Washington	fallow	Dactylis glomerata Taraxacum officinale Sorghum halepense Salsola kali	none
Wisconsin	fallow	fallow	none

unit. Roots were separated from the soil by sieving through a U.S.D.A. Standard Testing Sieve No. 10 (2 millimeter opening) and fixed in FAA. The sieved soil samples were kept in plastic bags at 2 C pending their use for spore population estimates, and as inocula for trap plants.

Soil pH and nutrient status were evaluated as part of the NC-140 project (Table 2), the analyses being done by the Research-Extension Analytical Laboratory of the Ohio Agricultural Research and Development Center, Wooster, Ohio. Soil description information (Table 2) was supplied by each NC-140 cooperator.

Endogonaceous spores were extracted from 100 g subsamples of nine randomly selected soil samples per site by wet sieving, sucrose centrifugation (Walker, et al., 1982) and were preserved in FAA. Endogonaceous species present in these samples were identified and counted to determine total spore numbers and relative species abundance. Counts were made from spore samples suspended in water in a 60 mm diameter petri dish through a dissecting microscope. Five aliquots were counted per site. Counts are reported as means with related standard error. Voucher specimens have been placed in the herbaria of Iowa State University (ISC) and Oregon State University (OSC).

Root samples collected during the initial survey were cleared and stained and evaluated after Kormanik, et al. (1980). The apple roots were heavily pigmented and required 2-2.5 hr in the bleach solution. Roots were suspended in destaining solution in a petri dish, sections

Table 1.2 Soil type, soil pH and soil nutrient status at each NC-140 regional apple rootstock planting site sampled, August 1980

SITE	SOIL DESCRIPTION[a]	pH	SOIL NUTRIENT STATUS (mg/kg) P	K	CA	MG	MN	ZN	B	CEC[b]
Arkansas	Linker sandy loam (fine-loamy, siliceous, thermic Typic Hapludults)	5.7	6	75	455	78	80	1.4	0.26	7
California	Elder sandy loam (coarse-loamy, mixed, thermic Cumulic Haploxerolls)	6.5	187	480	2680	644	15	33.4	0.67	22
Georgia	Evard loam (fine-loamy, oxidic, mesic Typic Hapludults)	5.8	13	115	445	44	12	2.4	0.49	8
Illinois	Drummer silt loam (fine-silty, mixed, mesic Typic Haplaquolls)	5.6	8	107	2250	374	17	10.2	1.12	24
Indiana	Brookston silty clay loam (fine-loamy, mixed, mesic Typic Argiaquolls)	5.8	13	121	1405	393	25	5.0	0.53	14
Iowa	Clarion silt loam (fine-loamy, mixed, mesic Typic Hapludolls)	6.4	13	100	1990	431	12	3.8	0.39	17
Kansas	Muir silt loam (fine-silty, mixed, mesic Cumulic Haplustolls)	6.7	31	442	1807	264	8	6.5	0.49	13
Kentucky	Tilsit silt loam (fine-silty, mixed, mesic Typic Fragiudults)	6.4	5	83	1576	228	16	4.6	0.50	11
Massachusetts	Montauk loam (coarse-loamy, mixed, mesic Typic Fragiochrepts)	5.9	152	45	370	124	8	2.7	0.39	11
Michigan	Conover loam (fine-loamy, mixed, mesic Udollic Ochraqualfs)	5.3	26	88	590	95	18	3.9	0.71	11

Minnesota	Lesauk clay loam (sandy, mixed frigid Typic Haplaquolls)	5.9	41	129	2065	237	45	7.9	0.57	17
New York	Collamer silt loam (fine-silty, mixed, mesic Glossoboric Hapludalfs)	6.6	22	81	1735	225	27	7.8	0.53	12
Ohio	Wooster silt loam (fine-loamy, mixed, mesic Typic Fragiudalfs)	4.9	14	78	655	106	84	4.6	0.41	12
Oregon	Chehalis silty clay loam (fine-silty, mixed, mesic Cumulic Ultic Haploxerolls)	6.2	41	403	2290	752	22	7.9	1.69	25
Pennsylvania	Hagerstown silt loam (fine, mixed, mesic Typic Hapludalfs)	6.5	19	66	1380	149	58	4.0	0.32	11
Virginia	Groseclose silt loam (clayey, mixed, mesic Typic Hapludults)	5.6	11	135	580	120	34	5.2	0.78	8
Washington	Burch loam (coarse-loamy, mixed, mesic Aridic Haploxerolls)	5.6	39	212	775	140	11	12.4	0.12	7
Wisconsin	Longrie silt loam (coarse-loamy, mixed, frigid Entic Haplorthods)	6.6	65	124	1120	248	13	4.8	1.58	9

[a]Soil Survey Staff, 1975.
[b]Cation Exchange Capacity.

counted, and evaluated for fungal colonization through a dissecting microscope (4- 40x) and, if higher magnification was needed, through a compound microscope. Observations for individual trees were combined to give a mean per site class and intensity rating.

Pot cultures were established in the greenhouse in summer 1981 in a 1:1:1 (v/v/v) mixture of mixed soil from each site: calcined clay (Oil Dry Corp. of America): vermiculite. The mixed soil from each site was composed of portions of ten randomly selected samples shaken together. Sorghum (from seed) and Coleus (from stem cuttings) were used as trap plants. Standard round (30.5 cm) pots were used with 2 pots per site. Aluminum pans were used as saucers and pots plus saucers were placed on inverted 30.5 cm pots to prevent cross contamination. Cultures were arranged in a completely randomized design on a greenhouse bench. Pot cultures were maintained for six months. They received occasional dilute nitrogenous fertilizer until harvest. The soil was evaluated by wet sieving, sucrose centrifugation for fungal species. Root colonization was not assessed.

To compare infective potential among soils, seven two-fold dilutions of a mixed soil sample from each site were made in a dilution (similar to most- probable- number) (Porter, 1979) study with apple as the indicator host. Moisture content of soils was determined gravimetrically (range 3- 23% among soils). For each site, an amount equivalent to 50 g dry weight of soil was mixed with a similar amount of Clarion silt loam soil that had been fumigated, and shaken

vigorously for the 2^{-1} dilution. Half the total weight of this diluted soil was then added to an amount equivalent to 50 g dry weight of fumigated soil and shaken for the 2^{-2} dilution and so on to the 2^{-7} dilution.

Apple seedlings (open pollinated seeds from 'Golden Delicious') were grown in vermiculite and selected for uniformity at the first true leaf stage. Seedlings were transplanted into 6 cm white plastic pots containing a 1:1:1 (v/v/v) mixture of fumigated Clarion silt loam soil: calcined clay: vermiculite. At planting time, 5 g of the test soil were dribbled onto the roots. Five dilutions from each of the 18 sites were used (2^{-3} through 2^{-7}) with 5 replications of each dilution and a fumigated control treatment was included. Pots were placed on inverted 8 cm pots on the greenhouse bench in a completely randomized statistical design. Plants were fertilized fortnightly with low rates of nitrogenous fertilizer. After 4 months feeder roots were collected and preserved in FAA. These roots were cleared and stained (Kormanik, et al., 1980) and evaluated for presence or absence of colonization although intensity of colonization was not measured. Estimates of propagule numbers and confidence intervals were calculated using the formula and table developed by Stevens (page 66, Table VIII, Fisher and Yates, 1963).

Attempts to establish pure pot cultures of each fungal species were made with freshly extracted spores from either the original soil or from the mixed pot cultures. Spores were surface sterilized for three minutes in a 2% solution of Chloramine-T containing 200 ug/ml

streptomycin followed by three rinses in distilled water. Apple (open pollinated seeds of 'Golden Delicious') and sorghum were germinated in vermiculite and 1-12 spores placed directly onto the roots of each plant host when the the first true leaf had appeared. After inoculation, plants were grown in a medium of 1:1:1 (v/v/v) fumigated Clarion silt loam soil: calcined clay: vermiculite in 6 cm diameter white plastic pots. To prevent cross-contamination, pots were placed on inverted 8 cm pots with plastic petri dish (100 x 15 mm) halves as saucers. The initial phosphorus level of the media was 28 ug/g (Knudsen, 1980). Plants were fertilized fortnightly with dilute nitrogenous fertilizer and grown for 4 months. Root samples were collected and cleared and stained to assess mycorrhizal colonization. Soil samples from the pots were examined for presence of endogonaceous spores. Successful pure pot cultures have been deposited with N. C. Schenck, Department of Plant Pathology, University of Florida.

RESULTS

There was a wide range of mineral levels and cation exchange capacity among sites (Table 2). Phosphorus and zinc ranged from 5 to 187 mg/kg and 1.4 to 33.4 mg/kg, respectively. Soil pH ranged from 4.9 to 6.7 among sites.

All apple roots collected were endomycorrhizal (Table 3), those from sites in Virginia, Pennsylvania, Georgia, and Iowa being most heavily colonized. Roots from California and Washington were very lightly colonized. Arbuscules and vesicles were evident in roots from all sites except California where only vesicles were noted. There was no evidence of any ectomycorrhizal association with any roots. Within sites, root colonization showed little tree to tree variation and, hence, no apparent differences among rootstock clones and thus data are expressed as mean class and intensity of colonization per site. Regression analysis revealed that root colonization was inversely related to soil zinc levels ($r=0.66$ **). Colonization was usually inversely related to soil phosphorus levels but a few exceptions (high phosphorus, high colonization) obliterated statistical significance. A multiple regression analysis of root colonization left a linear residual after the effects of zinc and phosphorus were removed suggesting involvement of other factors which could not be isolated.

In the initial soil sievings, the number of endogonaceous species encountered at each site ranged from 3 to 8 (Table 4). The number of spores per 100 g dry weight soil ranged from 62±19 to 2151±199 among

Table 1.3 Mycorrhizal colonization of fine roots of apple collected at each NC-140 regional apple rootstock planting site, August 1980

Site	Number Root Pieces Examined[a]	Class[b,c]	Intensity[b,d]	Type[e]
Iowa- nursery stock	2000	3.2	1.8	v,a
Arkansas	205	3.4	1.6	a,v
California	136	1.1	1.0	v
Georgia	303	3.9	1.8	a,v
Illinois	145	2.2	1.6	v,a,s
Indiana	287	2.1	1.2	v,a
Iowa	532	3.8	1.7	v,a
Kansas	348	2.5	1.3	v,a
Kentucky	174	2.0	1.2	v,a
Massachusetts	333	3.6	1.7	a,v
Michigan	472	3.6	2.3	a,v
Minnesota	125	2.4	1.2	v,a
New York	374	3.5	1.5	v,a
Ohio	155	3.2	1.5	a,v,s
Oregon	980	3.0	1.6	a,v,s
Pennsylvania	530	3.9	1.9	a,v,s
Virginia	322	4.2	2.0	v,a
Washington	145	1.5	1.0	a,v
Wisconsin	59	2.4	1.4	v,a

[a] 2 cm sections.

[b] Evaluations made on per tree basis; data presented as means per site.

[c] Class 1= 0-5% of root length colonized
2= 6-25%
3=26-50%
4=51-75%
5=76-100%.

[d] Intensity 1=small infection sites, widely scattered
2=larger infection sites, uniformly distributed but rarely coalescing
3=solid colonization throughout root piece.

[e] v=vesicles, a=arbuscules, s=spores; listed in order of predominance.

Table 1.4 Endogonaceous fungi species identified, relative abundance of each species, and total spore counts for each NC-140 regional apple rootstock planting site sampled. Key- ● fungal species represents >50% of number of spores counted in soil samples from that site. ✪ fungal species represents 5- 50% of spores. • fungal species represents 1- 4.9% of spores. ★ fungal species represents <1% of spores. **PC** fungal species found only in pot culture of composite soil

Species \ Site	AR	CA	GA	IL	IN	IA	KS	KY	MA	MI	MN	NY	OH	OR	PA	VA	WA	WI	sites/ species
Glomus albidum Walker & Rhodes												✪							1
Glomus aranaceum (Thaxter)										PC									1
Glomus bitunicatum Walker & Miller				PC															1
Glomus caledonium (Nicol. & Gerd.) Gerd. & Trappe														✪					1
Glomus constrictum Trappe	✪		✪		✪	•	✪	✪	✪	•	✪		•	✪	✪	✪	✪	✪	15
Glomus epigaeum Daniels & Trappe															★				1
Glomus etunicatum Becker & Gerd.			PC			PC													2
Glomus fasiculatum (Thaxter sensu Gerd.) Gerd. & Trappe																		●	1
Glomus fragile (Berk. & Br.) Trappe & Gerd.														✪					1
Glomus geosporum (Nicol. & Gerd.) Walker															✪				1
Glomus 'globisporum'									PC										1
Glomus invermaium Hall																PC			1
Glomus maculosum Miller & Walker																		★	1
Glomus hyalosporum Walker & Miller			PC									PC				PC			3
Glomus macrocarpum Tul. & Tul.							•												1
Glomus manihotis Howeler, Sieverding & Schenk								PC											1
Glomus melanosporum Gerd. & Trappe				★															1
Glomus microcarpum Tul. & Tul.		✪				•										✪		★	4
Glomus monosporum Gerd. & Trappe														•		★		★	3
Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe			★	✪		★					•		●	✪	✪	✪			8
Glomus occultum Walker			PC	✪	PC	•											PC	PC	6
Glomus 'rigidicaulis'																		PC	1

	AR	CA	GA	IL	IN	IA	KS	KY	MA	MI	MN	NY	OH	OR	PA	VA	WA	WI	
Glomus stramentotinctum Walker & Miller												PC							1
Glomus sp.	●	✪	●	●	●	●		●	●	●	●				✪	●	✪		
Gigaspora calospora (Nicol.&Gerd.)Gerd.& Trappe	✪		PC		✪		•	✪	PC	✪	★	✪					✪	✪	11
Gigaspora coralloidea Trappe, Gerd.& Ho	•								★			✪							3
Gigaspora dipapillosa Koske & Walker	PC																		1
Gigaspora erythropa Koske & Walker									PC			PC							2
Gigaspora gigantea (Nicol.&Gerd.)Gerd.& Trappe	★												•			★			3
Gigaspora gilmorei Trappe & Gerd.								PC	✪										2
Gigaspora heterogama (Nicol.& Gerd)Gerd.& Trappe			✪																1
Gigaspora margarita Becker & Hall												PC							1
Gigaspora pellucida Nicol.& Schenck									PC										1
Gigaspora reticulata Koske, Miller & Walker									★										1
Gigaspora rosea Nicol.& Schenck			•																1
Gigaspora sp.												★			•			★	
Acaulospora bireticulata Rothwell & Trappe										✪									1
Acaulospora scrobiculata Trappe			•																1
Acaulospora spinosa Walker & Trappe			PC																1
Acaulospora sp.																		★	
Sclerocystis rubiformis Gerd.& Trappe												●					✪		2
Sclerocystis sinuosa Gerd.& Bakshi	★						●												2
Sclerocystis sp.		✪						•											
Species/Site (soil sieving)	6	3	6	4	3	5	4	4	5	4	4	5	3	5	6	6	4	8	
Total Spores/100g soil/Site	1502 ±141	62 ±9	112 ±57	827 ±121	150 ±21	836 ±76	1083 ±179	318 ±47	885 ±106	276 ±62	278 ±21	350 ±70	161 ±22	138 ±31	241 ±16	676 ±130	2151 ±199	1232 ±204	
Additional Species Encountered in Composite Pot Cultures	1	0	5	1	1	1	0	2	4	1	0	4	0	0	0	2	1	2	

sites. Twenty-seven distinct spore types were identified from sievings of collected soil with 2 of these (Gigaspora reticulata Koske, Miller and Walker, Glomus maculosum Miller and Walker sp. ined.) being undescribed at the time of the survey. Species most commonly encountered were Glomus constrictum, Glomus mosseae, and Gigaspora calospora. Small, yellow to brown spores (Glomus spp.) were relatively abundant at several sites. Soil phosphorus or soil zinc levels were not correlated with the number of species or the number of spores among sites.

Not all fungal species present in survey soil reappeared in pot cultures with Sorghum and Coleus (Table 4). No new Sclerocystis spores were found and very few cultures produced new spores of Glomus constrictum. Fourteen species which had not been seen as spores in original soil sievings appeared in abundance in these pot cultures (Table 4) with six of these (Glomus bitunicatum Walker and Miller sp. ined., Glomus hyalosporum Walker and Miller sp. ined., Glomus manihotis Howeler, Sievarding, and Schenck sp. ined., Glomus stramentotinctum Walker and Miller sp. ined., Gigaspora dipapillosa Koske and Walker sp. ined., and Gigaspora erythropa Koske and Walker sp. ined.) being undescribed at the time. The taxonomic status of the remaining spore types, Glomus aranaceum, Glomus 'rigidicaulis', and Glomus 'globisporum' remains unsettled.

Infective potential of soils with apple, estimated by the dilution study, ranged from 0.6 to 23 propagules per g dry weight soil (Table 5). The three sites with highest estimated infective potential

Table 1.5 Estimates of infective potential of survey collected soil

Site	Dilution Study: estimated density of infective propagules per gram soil	2-sided 95% confidence intervals: lower	upper
Arkansas	8.3	4.5	15.4
California	0.6	0.3	1.2
Georgia	18.1	9.7	33.6
Illinois	4.3	2.3	7.9
Indiana	1.9	1.0	3.5
Iowa	23.0	11.2	38.6
Kansas	3.1	1.7	5.8
Kentucky	4.3	2.3	8.1
Massachusetts	2.3	1.2	4.2
Michigan	5.1	2.7	9.4
Minnesota	8.3	4.5	15.4
New York	5.9	3.2	11.0
Ohio	5.1	2.7	9.4
Oregon	8.3	4.5	15.4
Pennsylvania	5.9	3.2	11.0
Virginia	14.6	7.8	27.1
Washington	1.3	0.7	2.4
Wisconsin	5.1	2.7	9.4

were Iowa, Georgia, and Virginia. Soil infective potential was correlated (r=0.71 **) with colonization of survey collected roots.

There was no correlation between spore numbers in soil (based on counts, Table 4) and soil infective potential (based on dilution study, Table 5). Because spore counts were made on 100 g soil samples, it was not possible to compare counts directly with estimates from the dilution study, but rankings between studies could be compared (Spearman rank correlation) (Steel and Torrie, 1980). From this type of comparison, Georgia ranked 17th in spore numbers but 2nd in infective potential. Washington ranked 1st in spores numbers but only 17th in infective potential. However, the site had been fumigated with methyl bromide prior to planting. California ranked last in both studies. Despite these, the ranges of the estimates considering all sites were similar, i.e. 62 to 2151 (spore counts) and 60 to 2300 (dilution study).

Fungal species found in the initial survey and mixed cultures were tested for colonization with apple and sorghum (Table 6). Apple was of primary interest but sorghum was used for comparison because it is commonly used as a host plant for pot cultures. In general, fungal species which colonized sorghum also successfully colonized apple and those not proved mycorrhizal with sorghum failed to become mycorrhizal with apple. No isolate of Glomus constrictum and no Sclerocystis species produced viable cultures with either host. Some isolates of Gigaspora calospora formed mycorrhizae while others did not. Some

Table 1.6 Successful pure pot cultures of endogonaceous fungi with apple (A) and/or sorghum (S) as plant host. Plus (+) indicates abundant spore formation

Species \ Site	AR	CA	GA	IL	IN	IA	KS	KY	MA	MI	MN	NY	OH	OR	PA	VA	WA	WI
Glomus albidum Walker & Rhodes												S+/A+						
Glomus aranaceum (Thaxter)																		
Glomus bitunicatum Walker & Miller				S+/A+														
Glomus caledonium (Nicol. & Gerd.) Gerd. & Trappe														A				
Glomus constrictum Trappe																		
Glomus epigaeum Daniels & Trappe															S			
Glomus etunicatum Becker & Gerd.			S+/A+			S+/A+												
Glomus fasiculatum (Thaxter sensu Gerd.) Gerd. & Trappe																		
Glomus fragile (Berk. & Br.) Trappe & Gerd.																		
Glomus geosporum (Nicol. & Gerd.) Walker																		
Glomus 'globisporum'																		
Glomus invermaium Hall																S+/A+		
Glomus maculosum Miller & Walker																		S/A
Glomus hyalosporum Walker & Miller			S+/A+									S+/A+				S+/A+		
Glomus macrocarpum Tul. & Tul.																		
Glomus manihotis Howeler, Sieverding & Schenk								S+/A+										
Glomus melanosporum Gerd. & Trappe																		
Glomus microcarpum Tul. & Tul.																		
Glomus monosporum Gerd. & Trappe																		
Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe			S+/A+			A+							S/A+	S/A+		S+/A+		
Glomus occultum Walker				A+	S/A	A											S+/A+	
Glomus 'rigidicaulis'																		S/A

	AR	CA	GA	IL	IN	IA	KS	KY	MA	MI	MN	NY	OH	OR	PA	VA	WA	WI
Glomus stramentotinctum Walker & Miller												S+/A+						
Glomus sp.																		
Gigaspora calospora (Nicol.&Gerd) Gerd.& Trappe	S+/A+						S/A				A	S/A						S/
Gigaspora coralloidea Trappe, Gerd.& Ho	S/A+																	
Gigaspora dipapillosa Koske & Walker	S/A																	
Gigaspora erythropa Koske & Walker									S+/A+			S+/A+						
Gigaspora gigantea (Nicol.&Gerd.) Gerd.& Trappe																		
Gigaspora gilmorei Trappe & Gerd.								A										
Gigaspora heterogama (Nicol.& Gerd) Gerd.& Trappe																		
Gigaspora margarita Becker & Hall												A+						
Gigaspora pellucida Nicol.& Schenck									S+/A+									
Gigaspora reticulata Koske, Miller & Walker									S/A									
Gigaspora rosea Nicol.& Schenck																		
Gigaspora sp.																		
Acaulospora bireticulata Rothwell & Trappe																		
Acaulospora scrobiculata Trappe																		
Acaulospora spinosa Walker & Trappe			S+/A+															
Acaulospora sp.																		
Sclerocystis rubiformis Gerd.& Trappe																		
Sclerocystis sinuosa Gerd.& Bakshi																		
Sclerocystis sp.																		

isolates of Glomus mosseae appeared more infective with apple than with sorghum.

DISCUSSION

The sites sampled in this survey encompass most of the diversity of soil type and nutrient status found in apple production regions. The availability of mineral nutrients to the apple trees at each site is influenced by factors such as soil texture and parent material, interactions among elements, root geometry, and mycorrhizae. Soil fertility is thought to be an important factor governing the presence and efficacy of mycorrhizae (Hayman, 1982). The negative correlation between soil zinc level (and the trend with phosphorus level) and root colonization confirms that, with apple, the presence of colonization is influenced by fertility but it is impossible to say how closely the presence of colonization and the efficacy in nutrient uptake are correlated. Mycorrhizal associations have been reported to be relatively self-regulating (Hayman, 1982). Thus if the association enhances uptake of P or other immobile elements, it is present, and if such elements are readily available their internal concentrations in the plant reduces mycorrhizal colonization (Gerdemann, 1968; Sanders, 1975; Jensen and Jakobsen, 1980; Hayman, 1982).

Root colonization among rootstock clones at each site was not significantly different and there was no evidence from this survey that the tendency to form mycorrhizae varied with clone. Root colonization among sites did differ significantly. Buwalda, et al. (1982) suggested that roots reach a maximum colonization level dependent on the interaction of host, endophyte, and environment.

This may explain why there was less colonization at some sites in the roots collected in the survey than in those collected before field establishment. The correlation between root colonization and host growth stimulation is not direct, but it is known that some colonization is required for a host growth response (Buwalda, et al., 1982). A single sampling of roots for assessment of fungal colonization is of limited value because the system is dynamic with both host and fungus growing. Responses of the various rootstocks to similar levels of colonization may differ but these determinations were not within the scope of the survey.

The observation that several species (3 to 8) of mycorrhizal fungi were present at each site complicates interpretation of their role in nutrient uptake. Greenhouse studies have indicated that fungal species may differ in colonization and efficacy depending on soil fertility and plant host (Plenchette, et al., 1983a; Plenchette, et al., 1983b). In this study, Glomus species were more widespread and produced more spores than Gigaspora, Acaulospora, or Sclerocystis. Spores of Acaulospora and Sclerocystis were rarely encountered.

There were no apparent patterns in geographic distribution of these fungi. Agricultural and horticultural soils have numerous opportunities to acquire non-indigenous fungal endophytes. Transplanting mycorrhizal nursery stock disseminated at least one species to field locations. At each site the variation in spore numbers among subsamples was small. This differs from the findings of Walker, et al. (1982) who found uneven spatial distribution of species

and spore numbers in newly established poplar plantations subjected to similar cultivation. Comparison of total spore numbers sieved from soil of the different sites should be viewed with some caution, as there were many different soil types involved and the wet sieving/ sucrose centrifugation procedure may not extract equal percentages of spores from each soil type (Walker, et al., 1982). Thus, the numbers should be considered as minima rather than as absolute values.

The fourteen species that were not observed in original soil samples but which appeared in mixed pot cultures were probably present in root fragments or as mycelium, rather than as spores at the time of sampling. Walker, et al. (1982) reported uneven distribution of endogonaceous spores within a site through time. There is evidence that this is so in this survey and that the single sample may have resulted in misrepresentation of the fungal populations.

The apple trees sampled in this survey were completing their first seasons growth on site. The fungal species available to the trees were those present either in the nursery or at the planting site. The population of endophytes at the site when the apple trees were planted was determined by the past vegetation in the field. After one years growth of apple the fungal populations would also be influenced by association with apple. Future sampling of sites would be more likely to reflect associations with apple, when spore counts might correlate more closely with root colonization and soil dilution estimates. Schenck and Kinloch (1980) found species composition changed with both the time and the crop growing at the site.

Mosse and Bowen (1968) reported that cultivated soils contain more numerous and diverse endogonaceous fungal populations than soils under natural vegetation. The evidence from this survey contradicts that conclusion. The Arkansas site was under natural vegetation for 20 years before establishing the apple planting and there were at least 5 different fungal species present with spore counts being second highest among all sites. Levels of P and Zn in the soil were very low at this location. Conversely, the intensively cultivated California site contained the least spores, fewest species, and highest fertility.

Very little is known regarding within site or within root competition among fungal species. It was not possible to identify the fungi from root colonization characteristics. In soils with fertility sufficiently high that mycorrhizae are not essential for plant survival, infectivity of the fungus may be more important that symbiotic efficacy (Bowen, 1980). Highly infectious but ineffective species may, under such conditions, be selected for over less infectious, more effective fungi (Bowen, 1980). Greenhouse pot cultures of mixed soil from each site with sorghum and coleus as trap plants, and pure pot cultures on sorghum and apple inoculated with spores, showed large differences in spore formation and root colonization among fungal species. Generally, in the pot cultures small spored species formed many more spores than did large spored species. There aree three likely explanations for why some species failed to establish mycorrhizae with apple or sorghum in this survey:

1) the species may not be mycorrhizal with these plant hosts, 2) the spores may not have been viable even though they appeared so, 3) the spores may have an extended dormancy requirement not broken in the conditions and time span used.

Spore numbers may reflect poorly the colonization potential of the soil (Hayman and Stovold, 1979; Porter, 1979). In this survey, overall spore numbers similarly failed to reflect soil infective potential. The correlation between soil infective potential (estimated by dilution with apple as the host plant) and root colonization of survey collected apple roots, and the lack of correlation of these with spore counts, indicates merit for the dilution technique in ecological studies. Such a technique is, however, more difficult than counting spores, and statistical confidence intervals widen as mean colonization potentials increase, making it difficult to separate soils statistically. The three sites with highest estimated infective potential (Iowa, Georgia, and Virginia) contained at least two fungal species that readily colonized apple in pure cultures.

There is no evidence that any of the sites were lacking in mycorrhizal inoculum potential. A better understanding of mycorrhizal associations could benefit apple tree production in the future. Many fungal species have been found to form endomycorrhizae with apple, and these should be evaluated not only for colonization potential, but also for efficacy, singly and in combination. The pure pot culture results suggest there are differences that may become important in

production schemes. For example, inoculation of tissue-cultured rootstocks may help the plant acclimatize to soil conditions. Reinoculation of an area after fumigation could be carried out with species most suited to the particular ecosystem. Nursery inoculation of clones would be easy and efficient and could lead to dissemination of suitable isolates.

There was no evidence of any ectomycorrhizal association with apple roots, and this concurs with the findings of Greene, et al. (1982).

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SECTION 2.

DIFFERENTIAL COLONIZATION AND EFFICACY OF ENDOMYCORRHIZAL FUNGI WITH APPLE SEEDLINGS AT TWO PHOSPHORUS LEVELS

Differential colonization and efficacy of endomycorrhizal fungi with apple seedlings at two phosphorus levels

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ABSTRACT

Six species of vesicular-arbuscular mycorrhizal fungi originally isolated from orchard soils were evaluated at low phosphorus (approximately 30 mg/kg) and high phosphorus (approximately 180 mg/kg) for root colonization and effects on growth of apple (Malus domestica Borkh.) seedlings. Fungal species evaluated were Glomus mosseae (Nicol. and Gerd.) Gerdemann and Trappe, Glomus maculosum sp. ined. Miller and Walker, Glomus manihotis sp. ined. Howeler, Sievarding, and Schenck, Gigaspora calospora (Nicol. and Gerd.) Gerdemann and Trappe, Glomus bitunicatum sp. ined. Walker and Miller, and two isolates of Glomus occultum Walker. Trees grew larger and had higher leaf P concentrations at high soil P than at low soil P regardless of mycorrhizae. At low P, fungal species differed in percent root length colonized, intensity of colonization, types of colonization structures formed, and number of spores produced. Fungal species also differed in effects on apple tree growth. At harvest, 5 treatments were taller than the non-mycorrhizal control trees and two were not. Stem diameter and total dry weight were increased by only three treatments. Root colonization was correlated with final height ($r=0.75$ **). At high P, no treatment resulted in greater than 5% of root length colonized and endogonaceous fungi had little influence on plant growth.

INTRODUCTION

Apple (Malus domestica Borkh.) is known to form vesicular-arbuscular mycorrhizae (VAM) with members of the genera Glomus, Gigaspora, and Acaulospora (Endogonaceae) (Miller, et al., 1983).

In common with many other plants, apple trees have greater mycorrhizal colonization in soils with low levels of available phosphorus (P) than in fertile soils and growth stimulation by mycorrhizal fungi is greatest where soils are low in nutrients (Covey, et al., 1981; Hoepfner, et al., 1983). The fungal symbionts explore the soil unreached by roots, thus giving the plant access to otherwise untapped sources of P (Hattingh, et al., 1973; Mosse, 1973a; Mosse, et al., 1973; Sanders and Tinker, 1971; Sanders and Tinker, 1973). Micronutrient uptake may also be aided by mycorrhizae (Gerdemann, 1964; Warcup, 1975; Daft and Hacskaylo, 1977; Cooper and Tinker, 1978; Lambert, et al., 1979a) and this has been shown for zinc with apple by Benson and Covey (1976). As soil P levels increase, root colonization decreases, and mycorrhizae may not then be necessary for satisfactory apple tree growth (Hoepfner, et al., 1983).

Species of endomycorrhizal fungi have been reported to lack host-specificity (Molina, et al., 1978), and although it is possible that specificity does occur (Rose, et al., 1979), it is probable that most endomycorrhizal fungi will be capable of symbiosis with most endomycorrhizal plants to at least some degree (Hayman, 1982).

Despite this lack of specificity, the mycorrhizal dependency

(Gerdemann, 1975) of a plant may vary with the species of fungus or with soil P level (Pope, et al., 1983), and this may be interpreted as a kind of functional host-specificity. Greenhouse studies with apple comparing two (Benson and Covey, 1976) and six (Plenchette, et al., 1982) fungal symbionts suggest different efficacies among symbionts. In a field study, Plenchette, et al. (1981) compared preinoculated plants with controls that became mycorrhizal naturally after outplanting, and attributed growth differences to the species of symbiont. The purpose of this study was to determine if different endomycorrhizal endogonaceous species isolated from around apple tree roots would have different effects on the growth of apple seedlings at the same stage of colonization at two levels of available phosphorus.

MATERIALS AND METHODS

Fungal Species

The fungi selected for this study were originally isolated from beneath young apple rootstocks in orchards in several different parts of the U.S.A. (Miller, et al., 1983). Soil from each site was used to inoculate Sorghum and Coleus to establish pot cultures (Gilmore, 1968), and these were maintained for 6 months until spores were extracted by centrifugation/sugar flotation (Walker, et al., 1982). Approximately 200 spores of each species were selected, surface sterilized by immersion in Chloramine T (Mosse, 1962) and streptomycin, and placed around the roots of non-mycorrhizal apple in a medium of 1:1:1 v/v/v methyl bromide fumigated Clarion silt loam soil, calcined clay (Oil Dry Corp. of America) and vermiculite. After three months, cultures were checked for purity, and extracted spores were used for this experiment.

The following six Glomus spp., and a Gigaspora sp. (A to G) were used in the experiment, with a non-mycorrhizal control (H). Samples of each species have been preserved in the collection of C. Walker, and each isolate given an accession number (Walker #). Prepared microscope slides have been deposited by the senior author in the herbaria of Iowa State University (ISC) and Oregon State University (OSC).

Treatment A - a non-sporocarpic form of Glomus mosseae (Nicol. and

Gerd.) Gerdemann and Trappe (1974) from the Ohio Agricultural Research and Development Center, Wooster, Ohio (Walker #502)

Treatment B - an undescribed Glomus sp., to be named Glomus maculosum sp. ined. Miller and Walker (manuscript in preparation), from the University of Wisconsin Peninsular Experiment Station, Sturgeon Bay, Wisconsin (Walker #765)

Treatment C - a previously undescribed species, soon to be named Glomus manihotis sp. ined. Howeler, Sievarding, and Schenck from the University of Kentucky Horticulture Research Farm, Princeton, Kentucky (Walker #647)

Treatment D - Gigaspora calospora (Nicol. and Gerd.) Gerdemann and Trappe (1974) from the New York State Agricultural Experiment Station, planting located near Sodus, New York (Walker #757)

Treatment E - an undescribed Glomus sp., to be named Glomus bitunicatum sp. ined. Walker and Miller (manuscript in preparation), from the University of Illinois Horticulture Research Farm, Urbana, Illinois (Walker #760)

Treatment F - Glomus occultum Walker (1982) from Purdue University Horticulture Research Farm, West Lafayette, Indiana (Walker #761)

Treatment G - Glomus occultum Walker (1982) of western U.S.A. origin. The spores used in the initial inoculation for this culture were of an undescribed Glomus sp., with small, pale yellow-brown spores from the Tree Fruit Research Center, Washington State University, Wenatchee, Washington (Walker #515). Although these spores appeared viable the Washington site had been fumigated with

methyl bromide and the inoculation attempt failed. Glomus occultum appeared in the culture, either as a fumigant survivor or as introduced into the soil from the roots of the nursery propagated apple rootstocks (Oregon Rootstock, Inc., Woodburn, OR) (Walker #763).

Phosphorus levels in soils from which the fungi originated were: Glomus mosseae, 14 mg/kg; Glomus maculosum, 65 mg/kg; Glomus manihotis, 5 mg/kg; Gigaspora calospora, 22 mg/kg; Glomus bitunicatum, 8 mg/kg; Glomus occultum (Indiana isolate), 13 mg/kg; Glomus occultum (western U.S.A. isolate), 40 mg/kg.

Trees

Apple seeds (open pollinated seeds from 'Golden Delicious') were germinated in vermiculite. Two weeks after emergence, the experimental seedlings were selected.

Potting Medium

Medium used was 25% methyl bromide fumigated Clarion silt loam soil: 35% calcined clay: 40% coarse vermiculite (v/v/v) in 1 liter white cylindrical polyethylene containers of 15 cm diameter. Initial soil nutrient levels in the Clarion silt loam soil were 48 mg/kg P, 100 mg/kg K, 1990 mg/kg Ca, 431 mg/kg Mg, 12 mg/kg Mn, 4 mg/kg Zn, and 0.4 mg/kg B. Soil pH was 6.3 and cation exchange capacity was 17%. Two phosphorus fertility levels were established. The high phosphorus

treatment received 5.0 g Osmocote 14-14-14 slow release fertilizer incorporated per pot. To correct for acidity induced by the Osmocote, 1.25 g $CaCO_3$ was added to each pot. The low P treatment received no added phosphorus but was treated every two weeks with NH_4NO_3, KNO_3, and $CaCO_3$ to give a total N and K content equivalent to that of the high P treatment.

Establishing the Experiment

Each apple seedling was inoculated by pipetting 1 ml of Ringer's solution containing approximately 200 spores directly onto the roots at planting. Controls each received 1 ml of Ringer's solution.

Plants were arranged on the greenhouse bench in a randomized complete block design with 5 blocks, each containing 16 plants (2 x 8 factorial). Plants were selected, planted, and inoculated June 20, 1982 and the experiment terminated 11 weeks later, by block, during the week of Sept. 12.

To prevent cross contamination among treatments, a 15 cm polyethylene saucer was placed under each pot. Plants were carefully watered as necessary with distilled water. The greenhouse was maintained at natural photoperiod and light levels with temperatures at 21-27 C by day and 19-20 C by night.

Measurements

Plant height (from soil level to growing tip) was measured fortnightly during the growing season. At harvest, stem diameter was measured 2 cm above soil level with a dial caliper. Roots were washed and spores extracted for quantification and evaluation of purity. Root fresh weight was recorded, and a small (1-2 g fresh weight) sample of feeder roots was randomly removed, weighed, and preserved in formaldehyde/ acetic acid/ alcohol (FAA) for later analysis of VAM's. The remaining root was oven-dried for 72 hr at 60 C for dry weight determination. A regression equation was used to estimate the oven dry weight of the removed sample and total dry weight obtained by addition. Dry weights of stem and leaves were determined and shoot to root ratios calculated. The preserved root samples were cleared and stained, and root colonization and type of fungal structures evaluated (Kormanik, et al., 1980).

Leaf, stem, and root samples were ground to pass a 40 mesh screen (375 micrometer openings), and dry-ashed for elemental analyses. Leaf tissue was analyzed for total N, P, K, Ca, Mg, Zn, Mn, Cu, and Fe levels. Stem and root tissue were analyzed for total P content. N content was analyzed using the Kjeltec System Micro-Kjeldahl procedure with selenic sulfuric acid digestion (Wall and Gehrke, 1975; Isaac and Johnson, 1976). Phosphorus was analyzed by the vanadomolybdophosphoric HCl procedure and evaluated spectrophotometrically. Other elements were analyzed by atomic

absorption spectrophotometry.

Soil pH was determined in a 0.01 M $CaCl_2$ solution. Soil phophorus was extracted by the Bray 1 technique and analyzed spectrophotometrically with the method of ascorbic acid color reaction (Knudsen, 1980).

Statistical Analyses

Data were analyzed by General Linear Models Procedure, Statistical Analysis System (Goodnight, 1979). Statistical analyses among fungal treatments within fertilizer were by single degree of freedom orthogonal contrasts (Steel and Torrie, 1980) of each fungal treatment with the non-mycorrhizal control.

RESULTS

Mean soil P level in low P soil mix was 28 mg/kg at the beginning of the experiment and declined to 22 mg/kg at the conclusion of the study. Mean soil P levels in high P soil mix were six to ten times that of low P soil ranging from 160 mg/kg at the beginning to 218 mg/kg at the conclusion of the experiment. There was much more variation among samples in high P soils than in low P soils due to the occurrence of particles of slow release fertilizer in some samples.

The average total dry weight of trees at low P was 6.0 g and at high P was 12.0 g. This doubling was typical of the differences found between fertility levels for other growth parameters. Leaf P levels of low P trees were 0.11% while high P trees averaged 0.28%. The low P trees were in the deficiency range defined by Shear and Faust (1980). Root colonization by mycorrhizal fungi, intense in low P trees, was almost absent in high P trees.

The simple effects of fungal treatment and P level were not independent for any of the growth parameters. Fungal treatments resulting in superior plant growth at low P did not perform similarly at high P. The effects of fungal treatments on plant growth are therefore presented by P fertility level.

At high P, the non-mycorrhizal control trees yielded average growth responses whereas in the low P treatment, they produced the smallest growth responses. Consequently, at high P, few contrasts of fungal treatment gave significant increases compared with non-

mycorrhizal controls even though the overall F was highly significant.

Shoot Height

Low P. There were no differences in shoot height among treatments during the first nine weeks (Table 1A) but by the 11th week the fungi in treatments A, B, C, E, and F produced plants taller than the non-mycorrhizal controls (H). Average final heights ranged from 21.6 cm (H) to 51.1 cm (C).

High P. Treatment affected plant height through week 7 but not at week 9 or 11 (Table 1B). Treatments B and D were taller and treatment C shorter than the non-mycorrhizal control at weeks 3, 5, and 7. Average final heights ranged from 53.0 cm (C) to 74.8 cm (B) with controls averaging 66.9 cm tall.

Plant Dry Weight

Low P. Total dry weight was correlated with final height (r=0.85 **) and was increased by three treatments (A, C, F) (Figure 1A). Total dry weights ranged from 3.71 g (H) to 7.99 g (F).

Shoot dry weight was correlated with final height (r=0.91 **) and the fungi influenced dry weight of leaves, stems, and consequently, shoots (Figure 1A). Four treatments (A, B, C, F) increased shoot dry weight when contrasted with the non-mycorrhizal control.

Root dry weight (Figure 1A) was not affected by treatment.

Table 2.1 Effect of fungal treatments on height and stem diameter of greenhouse grown apple seedlings. 1982

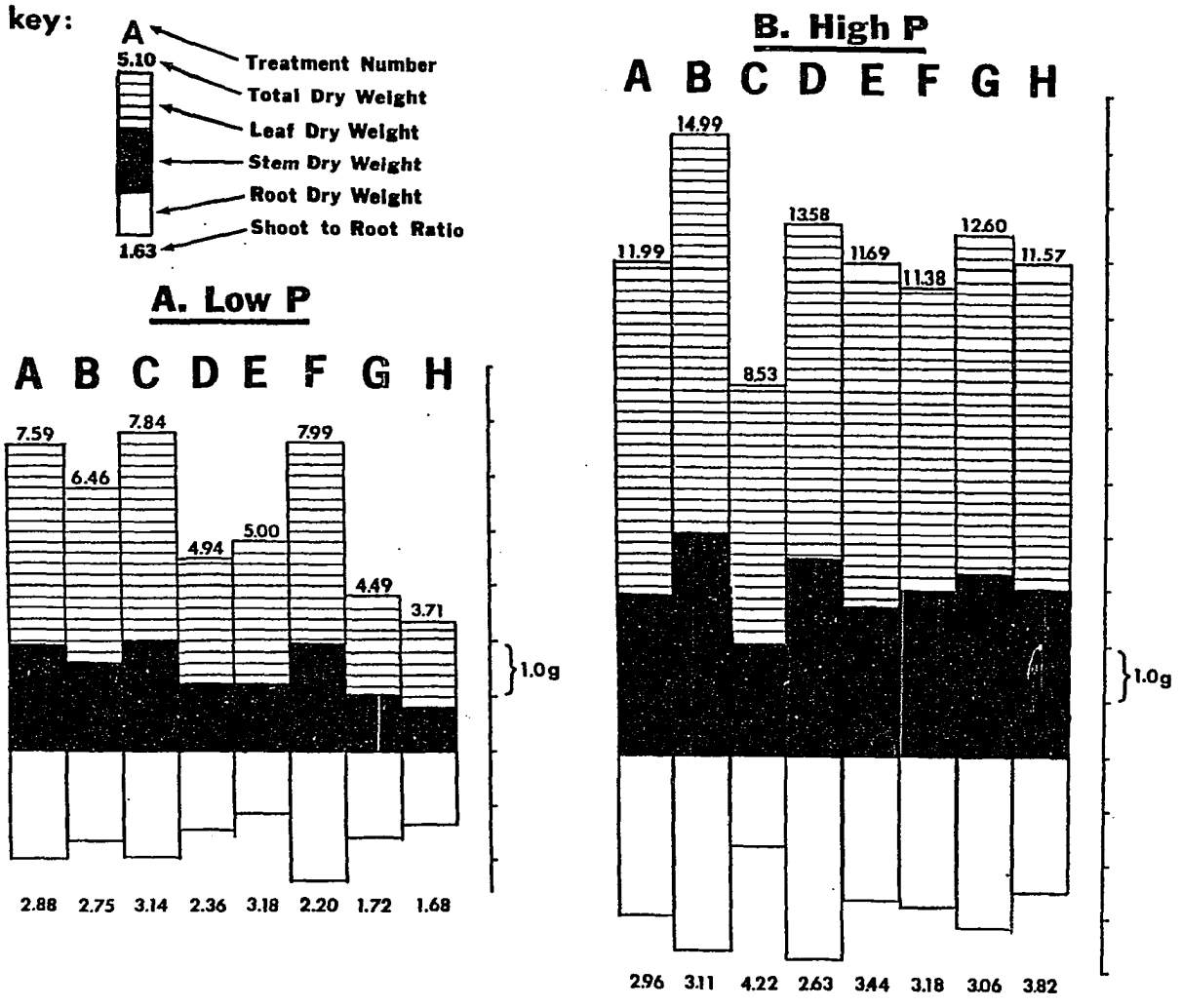
A. Low P

	Plant height (cm)					Stem diameter (mm)
Treatment	Week 3	Week 5	Week 7	Week 9	Week 11	Week 11
A	4.0	9.2	24.5	35.5	46.7	4.20
B	4.0	7.9	21.8	34.3	42.3	3.63
C	4.0	7.6	22.9	36.1	51.1	4.18
D	4.3	8.2	20.5	29.5	34.8	3.48
E	4.0	7.3	18.0	29.3	42.5	3.45
F	3.8	9.3	24.3	36.1	44.4	4.00
G	3.4	8.3	18.5	24.5	26.3	3.53
H	4.1	6.6	15.5	20.5	21.6	3.28
std. error	0.4	0.8	2.2	4.0	5.0	0.20

B. High P

	Plant height (cm)					Stem diameter (mm)
Treatment	Week 3	Week 5	Week 7	Week 9	Week 11	Week 11
A	4.9	15.0	32.8	42.8	59.4	4.90
B	5.8	17.7	41.3	56.1	74.8	5.48
C	2.4	7.5	20.9	36.4	53.0	4.16
D	5.3	16.9	39.2	50.0	67.6	5.38
E	3.2	9.4	28.9	41.9	56.3	4.60
F	4.2	12.1	30.1	43.0	60.4	5.03
G	4.0	13.1	33.1	45.8	63.5	4.83
H	3.9	12.0	30.2	45.7	66.9	4.82
std. error	0.5	1.6	3.1	4.0	5.1	0.20

Figure 2.1 Effect of fungal treatments on dry weight (total, leaf, stem, root) and shoot to root ratio of greenhouse grown apple seedlings. 1982. Standard errors: A. Low P Total Dry Weight 1.0; Leaf Dry Weight 0.4; Stem Dry Weight 0.3; Shoot Dry Weight 0.7; Root Dry Weight 0.3; Shoot to Root Ratio 0.3; B. High P Total Dry Weight 1.2; Leaf Dry Weight 0.5; Stem Dry Weight 0.4; Shoot Dry Weight 0.9; Root Dry Weight 0.4; Shoot to Root Ratio 0.4


key:
A
5.10
1.63
Treatment Number
Total Dry Weight
Leaf Dry Weight
Stem Dry Weight
Root Dry Weight
Shoot to Root Ratio
A. Low P
A B C D E F G H
7.59
6.46
7.84
4.94
5.00
7.99
4.49
3.71
1.0g
2.88 2.75 3.14 2.36 3.18 2.20 1.72 1.68
B. High P
A B C D E F G H
11.99
14.99
8.53
13.58
11.69
11.38
12.60
11.57
1.0g
2.96 3.11 4.22 2.63 3.44 3.18 3.06 3.82

Shoot to root ratio (SRR) (Figure 1A) ranged from 1.68 to 3.14. Treatments A, B, C, E had larger SRR than the non-mycorrhizal control.

High P. Total dry weight was not as highly correlated with final height (r=0.63 **) as in the low P treatment. Total dry weight ranged from 8.53 g to 14.99 g (Figure 1B). Only treatment B was larger than the non-mycorrhizal control.

Shoot dry weight also was not as highly correlated with final height (r=0.68 **) as it was in the low P treatment. Treatment did not influence shoot or leaf dry weight but did affect stem dry weight with treatment C having less than the non-mycorrhizal control.

Root dry weight was affected by treatment, with B and D increased compared with the control.

Shoot to root ratio was unaffected by treatment, ranging from 3.11 to 4.22 among treatments.

Stem Diameter

Low P. Stem diameter was highly correlated with total dry weight (r=0.91 **) and less correlated with final height (r=0.76 **). Treatment influenced stem diameter (Table 1A), treatments A, C, and F having larger diameters than the non-mycorrhizal control.

High P. Stem diameter was not well-correlated with total dry weight (r=0.69 **) and poorly correlated with final height (r=0.49 **). The effects of treatment on stem diameter was significant. Treatments B and D increased diameter and C reduced diameter compared

with the non-mycorrhizal control (Table 1B).

Plant Phosphorus

Low P. Leaf phosphorus concentrations ranged from 0.08 to 0.15% and were not affected by treatment (Table 2A). Symptoms characteristic of P deficiency (e.g., purplish leaf discoloration, reduced leaf size, and inhibited growth) were evident in some trees by week 9. Percentage P in the leaves was not correlated with any measured response. Stem P ranged from 0.07- 0.12% with treatments C and E containing higher concentrations than the non-mycorrhizal control. Root P ranged from 0.08- 0.15% with treatment E containing a higher concentration than the control.

High P. Leaf phosphorus concentrations ranged from 0.24- 0.33% (Table 2B) with treatments A and C containing higher concentrations than the controls. Stem P (range 0.20- 0.25%) and root P (range 0.29- 0.39%) were not influenced by treatment.

Other Plant Nutrients

Low P. Concentrations of K, Ca, Mg, Cu, Fe, Mn, and Zn did not differ among treatments and were in acceptable ranges (Shear and Faust, 1980). The concentrations ranged as follows: K, 1.33- 1.66%; Ca, 0.70- 0.99%; Mg, 0.33- 0.42%; Cu, 5.5- 8.5 µg/g; Fe, 37.0- 73.4 µg/g; Mn, 20.7- 33.5 µg/g; Zn, 23.0- 34.9 µg/g.

Table 2.2 Effect of fungal treatments on phosphorus concentrations (leaf, stem, root) of greenhouse grown apple seedlings. 1982

A. Low P

Treatment	Leaf P (%)	Stem P (%)	Root P (%)
A	0.11	0.09	0.09
B	0.10	0.07	0.08
C	0.15	0.11	0.13
D	0.09	0.07	0.08
E	0.13	0.12	0.15
F	0.11	0.09	0.10
G	0.10	0.07	0.07
H	0.08	0.07	0.09
std. error	0.02	0.01	0.02

B. High P

Treatment	Leaf P (%)	Stem P (%)	Root P (%)
A	0.33	0.25	0.39
B	0.29	0.20	0.20
C	0.32	0.24	0.32
D	0.24	0.22	0.30
E	0.24	0.24	0.32
F	0.28	0.22	0.29
G	0.25	0.24	0.31
H	0.27	0.21	0.34
std. error	0.02	0.01	0.04

Nitrogen ranged from 1.94- 2.63% and treatment A contained less N than the control. N concentrations were not correlated with differences in growth parameters.

High P. Concentrations of N, K, Ca, Mg, Cu, Fe, and Zn did not differ among treatments and were in acceptable ranges (Shear and Faust, 1980). The concentrations ranged as follows: N, 2.67- 2.94%; K, 1.27- 1.63%; Ca, 0.74- 1.00%; Mg, 0.36- 0.44%; Cu, 4.9- 7.2 µg/g; Fe, 47.5- 62.8 µg/g; Zn, 27.5- 33.1 µg/g.

Manganese concentrations ranged from 38.4- 62.3 µg/g and treatment C contained less Mn than the control.

Root Colonization

Low P. Treatments differed in the percent of roots colonized, intensity of colonization, types of structures found in the roots at time of harvest and number of spores produced (Table 3A) which were very consistent within a treatment at harvest. Root colonization (%) was better correlated with final height ($r=0.75$ **) than with total dry weight ($r=0.58$ **) or stem diameter ($r=0.57$ **). Some, but not all, fungal species appeared quite distinct in characteristics of structures formed in and on roots, and this was most notable in treatments A, C, and E (Table 3A). Typically, roots which were colonized were fine feeder roots and these were often colonized along their entire length. Occasionally, however, extension or secondary thickened roots contained fungal structures, usually vesicles.

Table 2.3 Characteristics of mycorrhizal associations of fungal treatments with roots of greenhouse grown apple. 1982

A. Low P

Treatment	Root colonization ratings: Class[a]	Intensity[b]	Spore production[c]	Root colonization characteristics
A	5.0	2.0	2.8	coarse hyphae wrapped around roots; no distinct vesicles
B	3.3	1.8	3.0	vesicles; hyphae in cortex
C	4.6	3.0	5.6	spores in roots rupturing epidermis; external hyphae on roots; spores common in sloughing cortex
D	3.0	1.0	3.2	colonization scattered; some arbuscules
E	4.6	3.0	3.5	very intense vesicles (spores?) in cortex; much external hyphae on roots; no arbuscules
F	4.0	1.8	3.5	external hyphae on roots; vesicles and arbuscules
G	3.0	1.5	4.0	external hyphae on roots; vesicles
H	1.0	0.0	1.0	no colonization

[a]Percentage root length colonized. 1= 0-5%; 2= 6-25%; 3= 26-50% 4= 51-75%; 5= 76-100%.

[b]Intensity of colonization. 0= none; 1= small, widely scattered; 2= larger infection sites, not coalescing; 3= solid infection.

[c]Spore production. 1= none; 2= less than 50; 3= 50-100; 4= 100-200; 5= many hundreds; 6= thousands.

Table 2.3 continued

B. High P

Treatment	Root colonization ratings: Class[a]	Intensity[b]	Spore production[c]	Root colonization characteristics
A	1.0	1.5	2.4	coarse hyphae wrapped around roots
B	1.0	1.0	2.5	very slight colonization
C	1.0	1.0	2.6	very slight colonization
D	1.0	1.0	2.0	colonization scattered; slight
E	1.0	1.0	1.2	some vesicles (spores?) in cortex
F	1.0	1.0	1.5	very slight colonization
G	1.0	1.0	2.0	some vesicles in cortex
H	1.0	0.0	1.0	no colonization

High P. No roots examined were colonized over more than 5% of their length (Table 3B) and many roots were not mycorrhizal. Treatment A had relatively more colonization than the others. Few spores were collected from soil sievings of any treatment (Table 3B).

DISCUSSION

Mycorrhizal associations of apple formed by the fungal species used in this study could not compensate for the large difference in P level between the low P and high P treatments. Trees grew larger at high P than at low P regardless of mycorrhizae. It is impossible to calculate the P threshold level for this soil with apple but it lies somewhere between the levels used in the experiment.

Studies with apple (Hoepfner, et al., 1983) and other plants (Menge, et al., 1978a; Menge, et al., 1978b) indicate that the benefits of mycorrhizae may be nullified by addition of P. Addition of P, however, is costly and due to the high P-fixing capacity of many soils and the low mobility of P ions, it is difficult to increase availability of this element. In field sites where apples are grown, P level variability may be as great as those in this study but the mean is in the low range (less than 30 mg/kg) (Miller, et al., 1983). Apple root colonization at low P (and Zn) sites often is intense (Miller, et al., 1983). In this study, intense colonization gave good growth stimulation, and this indicates that mycorrhizae may function to benefit growth of apple rootstocks in the field under such levels of available P.

In the low P treatment, there were differences in the ability of the various mycorrhizal fungi to enhance plant growth. Phosphorus concentrations of tissues did not usually differ, although two treatments, Glomus manihotis and Glomus bitunicatum, did result in

increased stem P, and trees mycorrhizal with Glomus bitunicatum also had increased root P. Perhaps this indicates a potential for increased growth stimulation under certain conditions. Fungal treatments resulting in larger trees had incorporated more P but the trees did not have higher P concentrations because of a dilution effect associated with growth.

Several species failed to improve plant growth though none resulted in reduced yields. These species must, therefore, have existed in a commensal relationship, under the conditions tested. Non-mycorrhizal trees initially grew as well as inoculated trees but slowed in rate by week 11, presumably when available P became limiting.

A factor undoubtably important in fungal efficacy, but one we did not quantify, is amount or intensity of hyphae distributed in the soil mix. If extramatrical hyphae are correlated with intramatrical hyphae (Sanders, et al., 1977; Tisdall and Oades, 1979; Sanders and Tinker, 1973) than differences among fungal species may be due to differences in volume of soil explored, because root colonization was correlated with plant growth. Zones of P deficiency, estimated to be 1-2 mm around a root (Rhodes and Gerdemann, 1975), would have occurred at low P and mycorrhizal fungi may explore soil at least 8 mm distant from the roots (Rhodes and Gerdemann, 1975). This distance may vary if different fungal species produce different amounts of hyphae.

Abbott and Robson (1979) and Abbott (1982) have concluded that colonization anatomy and pattern are diagnostic taxonomic characters

among selected fungal species. Distinct colonization characteristics occurred in apple roots with Glomus mosseae, Glomus manihotis, and Glomus bitunicatum at low P.

Roots were only examined at the conclusion of the study so early characteristics and dynamics of colonization were not determined. The number of roots originally inoculated were few, as seedlings were young, but colonization spread throughout the root system and apparently kept up with new root growth.

There was intraspecific variation seen in efficacy of Glomus occultum isolates at low P. Interpretation of this is complicated by the uncertain origin of one isolate. Graham, et al. (1982b) found intraspecific variation in efficacy of Glomus species related to amount of external hyphae which was influenced by edaphic factors, especially soil type and pH. The Indiana soil (Brookston silty clay loam, pH 5.8) from which Glomus occultum was isolated was more similar to the Iowa soil (Clarion silt loam, pH 6.3) used in this study in nutrient status, especially P, than to either the Washington soil (Burch loam, pH 5.6) or the Oregon soil (Chelalis silty clay loam, pH 6.2) from which the western U.S.A. isolate probably came (Miller, et al., 1983).

Mosse (1972) has stated that fungal effects on plant growth may depend more on interactions between a fungal strain and the soil than between a fungal strain and its host. In this study, 3 fungal species originally isolated from low P soils (Glomus mosseae, Glomus manihotis, Glomus occultum- Indiana isolate) increased apple stem

diameter at low P while 2 fungal species originally isolated from high P soils (Glomus maculosum, Gigaspora calospora) increased apple stem diameter at high P when contrasted with non-mycorrhizal trees.

High soil P levels are known to result in root P concentrations that inhibit mycorrhizae and reduce external hyphae of fungi in soil (Sanders, 1975). At high P, there was little evidence that inoculation with mycorrhizal fungi affected either plants or fungi. Growth of plants was similar among treatments (Table 1B) and it may be that differences in stem diameters at harvest reflected initial random variation in size among treatments.

Root distribution and geometry play a part in the ability of a plant to extract P from soil (Mosse, 1973b). Phenotypic variation was apparent in root characteristics of the apple seedlings in this study, especially in relative amounts of storage roots and fine feeder roots. These characteristics were not correlated with any particular growth or mycorrhizae effect but, because of random distribution among treatments, added to the variability of the experiment.

One of the problems in mycorrhizae research is ensuring uniform inocula potential. We used spores as inocula at a high rate per tree. There was no effect of spore size on inoculum potential, e.g. 200 large spores (Gigaspora calospora) did not colonize roots more intensely than 200 small spores (Glomus bitunicatum). We conclude that inoculum potential was adequate. Species tested received the same growth chamber conditions when in pot cultures in preparation for this study. Spores did not receive a cold treatment before use as

inoculum.

This study did not test the whole range of fungal species potentially mycorrhizal with apple. Those tested here were originally selected because they produced large numbers of spores in mixed cultures, greatly outnumbering other species in the same pots. This resulted in a preponderance of small-spored Glomus species. Many other fungal species should be tested with apple. Instead of selecting for species which produce abundant spores, it may be desirable to select from those producing fewer spores and more abundant extramatrical hyphae. These species may prove to be physiologically more active than those producing large numbers of spores, but they would require the use of root fragments rather than spores as a source of inoculum.

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SUMMARY AND CONCLUSIONS

1. Apple (Malus domestica Borkh.) rootstocks, regardless of clone, were endomycorrhizal in field plantings across a wide range of soil fertility. Root colonization percentage and intensity per site was negatively correlated with soil zinc and (at most sites) phosphorus. Infective potential of soils as estimated by soil dilution was well correlated with colonization of originally collected roots. This suggests mycorrhizae is a factor which may affect mineral nutrient uptake of apple trees in the field.

2. No geographic patterns in distribution of endogonaceous endophytes were apparent. A range of 3-8 fungal species were found among sites with Glomus being more common in number of species and number of spores than Gigaspora, Acaulospora, and Sclerocystis. Spore counts were a poor indicator of soil infective potential. The potential for root colonization by fungi was not lacking at any site but actual root colonization was regulated by factor(s) other than inoculum density.

3. Six fungal species isolated in the survey and evaluated at low phosphorus and high phosphorus as symbionts with apple seedlings in the greenhouse did not affect tree growth similarly at both P levels. In general, species isolated from low P soils were superior at stimulating tree growth at low P but not at high P while species isolated from high P soils were inferior symbionts at low P but were

not inferior at high P. Intraspecific variation in symbiont efficacy was apparent with Glomus occultum.

4. At low P, three fungal species, Glomus mosseae, Glomus manihotis, and Glomus occultum- Indiana isolate were superior symbionts in stimulating apple tree growth as compared to the non-mycorrhizal control trees. This demonstrates that despite the lack of host specificity in root colonization among these fungi there is a functional host specificity in efficacy.

5. Mycorrhizae could not substitute for the six-to-ten-fold difference between P levels and trees at high P averaged two times more growth than trees at low P. Phosphorus in available form (such as slow release fertilizer) apparently eliminated any benefit of mycorrhizae and reduced root colonization.

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Finally... an unedited page or two.

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APPENDIX A.

CENTRIFUGAL- FLOTATION TECHNIQUE FOR EXTRACTION OF ENDOGONACEOUS SPORES FROM SOIL (modified from Walker, et al., 1982)

1. Add desired amount of soil (I typically used 100 g) to bottom of 4000 ml polypropylene beaker. Fill beaker one-half full with tap water and swirl well with gloved hand. Allow the suspension to settle 15 seconds and decant through a U. S. Standard Testing Sieve No. 25 (710 micrometer opening) into another 4000 ml polypropylene beaker. Discard the debris on the sieve into the trash (save your plumbing).
2. Swirl the soil suspension in the second beaker and allow to settle 15 seconds. Decant through a U. S. Standard Testing Sieve No. 325 (45 micrometer opening). Save the material on the sieve. If the soil contains fine particles, the liquid will move slowly through the sieve. Slight jostling of the underneath side of the screen will speed water passage.
3. Wash material on the sieve into one area of the sieve via water spray from Tygon tubing attached to faucet. Place funnel into 50 ml polypropylene centrifuge tube and, using squirt bottle containing water, transfer material from sieve into centrifuge tube. Never fill the tubes so that they are more than one-fourth full of soil (i.e. use 2 tubes).
4. After balancing and stirring tubes, centrifuge for 3 minutes at 1700 rpm (begin timing as speed is reached).

5. Carefully decant the supernatant, fill the tubes with sucrose solution, balance, and stir. (Sucrose solution is made as follows: Add 700 ml H_2O to 1 liter flask, add sucrose to bring to 1 liter volume; requires some stirring time to go into solution.) Centrifuge for 15 seconds at 1700 rpm (begin timing as speed is reached).

6. Pour the supernatant through a U. S. Standard Testing Sieve No. 400 (38 micrometer opening). Rinse material on the sieve with water. Use squirt bottle containing Ringer's solution to transfer material into 10 cm diameter polystyrene petri dishes.

7. Examine dishes within the next few hours to avoid overgrowth of endogonaceous spores by contaminates.

APPENDIX B.

APPLE ROOT CLEARING AND STAINING PROCEDURE (modified from Kormanik, et al., 1980)

1. Place roots in 38x8 mm Tissue-Tek capsules at harvest and store in formaldehyde/ acetic acid/ alcohol (FAA) until cleared and stained. Fill capsules full but do not cram.
2. Take safety precautions as some reagents used in this technique are caustic.
3. Place Tissue-Tek capsules containing roots in 2000 ml beaker (fill beaker no more than one-half full). Cover with stainless steel screen cut to fit within beaker and weight screen down (I used inverted glass funnel). This will contain capsules and allow easy handling.
4. Cover to top of screen with 10% KOH and autoclave for 15 min at 20 psi in autoclave. Pour KOH down drain with lots of water.
5. Rinse roots with tap water at least 3 complete changes of water.
6. Immerse roots in alkaline H_2O_2 at room temperature until bleached. With apple, this takes 2- 2.5 hr; with sorghum, this takes 0.5 hr. The bleach solution is made as follows: 30 ml 30% H_2O_2 plus 9 ml NH_4OH (standard household ammonia) plus 1760 ml H_2O. Mix this solution up just before use. After use, it can be discarded down the drain with water.
7. Rinse roots with tap water at least 3 complete changes of water.
8. Acidify in 2.5% HCl for 3 minutes; drain in sink.

9. Stain 12 minutes in autoclave at 20 psi in acid fuchsin in a lactic acid/ glycerin mixture. To make one liter of solution: 875 ml 85% lactic acid plus 63 ml glycerol plus 62 ml H_2O plus 0.1 g acid fuchsin. This solution requires considerable mixing time and can be made up ahead of time and stored. It can be reused once but then loses staining effectiveness.

10. Remove roots from capsules, place roots in plastic petri dishes, and add destaining solution (staining solution without the acid fuchsin) to cover. The roots can be viewed immediately but are easier to evaluate after 1- 3 days in destaining solution.

11. Roots will store in destaining solution for several weeks.